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GROWTH EFFECT OF VARIOUS ANTIBIOTICS ON BABY PIGS FED SYNTHETIC RATIONS[1]

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and B. CONNOR JOHNSON

University of Illinois[4]

NESHEIM and Johnson (1950) reported that streptomycin increased the rate and efficiency of gain of baby pig pair-fed a soy-protein "synthetic milk" diet. While using a casein "synthetic milk" diet, Nesheim *et al.* (1950) reported no statistically significant response from the addition of aureomycin, streptomycin or penicillin when the animals were pair-fed. Using *ad libitum* feeding, Wahlstrom *et al.* (1950) found that aureomycin hydrochloride produced a significant growth response in the baby pig on an alpha protein "synthetic milk" diet, but had no effect on the number of coliform, lactobacilli or yeast cells present in the feces.

Numerous other investigators (e.g., Luecke *et al.*, 1950; Jukes *et al.*, 1950; Carpenter, 1950) have reported that antibiotics produce growth stimulation in weanling pigs. The present study was conducted to study further the mode of action of antibiotics and to compare the growth stimulating effects of chloromycetin and aureomycin when fed to baby pigs on "synthetic milk" diets.

Experimental Procedure and Results

Experiment I

Eight Chester White pigs were removed from their dams at 48 hours of age and were fed *ad libitum* the alpha-protein "synthetic milk" ration given in table 1. The technique of feeding and care of the animals was similar to that reported previously by Johnson *et al.* (1948). The pigs were divided into two groups of four pigs each. Group I received

[1] A preliminary report of this work was made before the American Institute of Nutrition meeting in Cleveland, May, 1951. Federation Proceedings, 10:397, 1951.

[2] Swine Division.

[3] Chloromycetin was supplied by the Parke Davis Company, Detroit, Michigan. through the courtesy of Dr. Orson D. Bird.

We would like to acknowledge the provision made by Lederle Laboratories Division, American Cyanamid Corporation, Pearl River, New York, for a grant-in-aid for partial support of our baby pig research.

Inositol was supplied by the A. E. Staley Manufacturing Company, Decatur, Illinois.

Methionine was supplied by Dow Chemical Company, Midland, Michigan, through the courtesy of Dr. Julius Johnson.

[4] Division of Animal Nutrition, Urbana.

the basal ration only, and group II received the basal ration plus 100 mg. of aureomycin hydrochloride per kg. of dry matter consumed. Fresh fecal samples were collected from pigs of each group, and total aerobic and total anaerobic bacterial counts were determined using blood agar plates.

Weighed fecal samples of 0.1 gm. were diluted to 100 cc. to give a concentration of 10^{-3}; further dilutions were then made from this

TABLE 1. COMPOSITION OF ALPHA-PROTEIN "SYNTHETIC MILK"

Ingredient[1]	Percent
Alpha protein	29.5
DL-methionine	0.5
Glucose (cerelose)	31.7
Lard	30.0
Mineral salts[2]	8.3

Vitamins added per liter

Thiamine	0.65 mg.	Biotin	0.01 mg.
Riboflavin	1.30 "	Folic acid	0.52 "
Pyridoxine	1.30 "	Ascorbic acid	0.01 "
Calcium pantothenate	7.80 "	Alpha tocopherol acetate	1.00 "
Inositol	26.00 "	2-Methyl-1, 4-napthoquinone	0.26 "
Nicotinic acid	2.60 "	Vitamin A	2000 I.U.
Choline chloride	130.00 "	Vitamin D	200 I.U.

[1] These materials, plus the fat soluble vitamins, were homogenized into a "synthetic milk" containing 13 percent solids and 4 percent fat. Vitamin B_{12} was injected intramuscularly at a level of 0.8 mcg. per kg. of body wt. per day.

[2] Same as Johnson *et al.* (1948) except that Ca lactate replaces $CaCO_3$ in equimolar amount.

concentration. Anaerobic bacteria were determined by pouring blood agar plates which were incubated in an anaerobic jar under nitrogen. After incubation, colonies were picked and placed into thioglycollate broth. From this, gram stains were made for microscopic identification of spore-forming bacteria. The modified Wilson-Blair medium as used by Sieburth *et al.* (1951) was also employed to determine *Clostridium perfringens*. In another trial to determine the presence of Clostridia in the feces of baby pigs, feces was streaked directly on blood agar plates which were incubated for 72 hours under carbon dioxide. Slides with simple stains were then made and examined microscopically. Other colonies were then picked to litmus milk with paraffin seal and $FeCl_3 + Na_2SO_3$ was added as indicator (method of Robinson and Stovall, 1939), in an attempt to produce stormy fermentation and discoloration. Duplicate tubes were boiled to destroy everything but spores.

At the close of the experiment all of the pigs in group II and the largest pigs in group I were sacrificed. Examination of the digestive tract and organs was made.

A summary of the results is presented in table 2. The average daily gain of the pigs fed aureomycin was significantly more rapid than was the average daily gain of the basal group. There was no difference in efficiency of gain. No difference was found in the total number of aerobic or of anaerobic bacteria present in the feces. No evidence of spore-forming bacteria or Clostridia was found in the feces of either the basal or aureomycin-fed pigs. Examination of the

TABLE 2. GROWTH RESPONSE OF BABY PIGS FED AUREOMYCIN (49-DAY TEST)

	Group I Basal	Group II Basal plus 100 mg. aureomycin per kg. dry matter consumed
No. of pigs	4	4
Av. initial wt., kg.	1.33	1.37
Av. final wt., kg.	13.54	17.26
Av. daily gain, lbs.	0.55	0.71*
Feed consumed per kg.	1.26	1.22

* Significant over Group I ($P<.04$).

digestive tract at the time of slaughter showed an increase in caecal size of the aureomycin-fed pigs. This approximately three-fold increase in caecal size was not accompanied by significant increase in weight of the digestive tract.

Experiment II

The purpose of this experiment was to compare aureomycin and chloromycetin when added to an otherwise nutritionally adequate "synthetic milk" diet.

Nine 2-day-old Chester White pigs were used in this experiment. They were fed the same basal ration as in experiment I. Group I received the basal ration only, group II received the basal ration plus 100 mg. of aureomycin per kg. of dry matter consumed, and group III was given the basal ration plus 100 mg. of chloromycetin per kg. of dry matter consumed. Fecal samples were taken as in experiment I, and bacterial counts were determined. Coliform and lactobacilli counts were determined on these samples by dilution methods employing Bacto-E C medium (Difco) for the *E. coli* counts, and Lactalysate

TABLE 3. GROWTH RESPONSE OF BABY PIGS FED AUREOMYCIN OR CHLOROMYCETIN (56-DAY TEST)

	Group I Basal	Group II Basal plus 100 mg. aureomycin per kg. dry matter consumed	Group III Basal plus 100 mg. chloromycetin per kg. dry matter consumed
No. of pigs	3	3	3
Av. initial wt., kg.	1.61	1.59	1.58
Av. 5-week gains, kg.	6.39	9.29*	9.61**
Av. final wt., kg.	20.93	22.73	23.56
Av. daily gain, lbs.	0.76	0.83	0.86
Feed consumed per kg. gain, kg.	1.22	1.21	1.23

* Significant over Group I ($P<.05$)
** Significant over Group I ($P<.04$)

broth (BBL) for the lactic counts. Coliform counts were also determined using violet red bile agar plates.

A summary of the total performance of each of the 3 groups of pigs is given in table 3. Both aureomycin-fed pigs and the chloromycetin group gained significantly faster than did the basal pigs. However, the greatest difference was noted during the fourth to the sixth week of the experiment, at which time the differences in average daily gain were statistically significant. During the last two weeks of the test there was little difference between groups.

The results of the bacteriological counts are presented in table 4. During the first 9 days there was a marked decrease in coliform organisms in the feces of the pigs receiving chloromycetin. By the sixteenth day this difference had disappeared, and there was no apparent effect at this time of either chloromycetin or aureomycin. Chloromycetin also appeared to decrease the lactic counts, although this decrease was not marked as in the case of the coliform.

TABLE 4. BACTERIAL COUNTS GIVING GREATEST DILUTION AT WHICH GROWTH OCCURRED

	Group I Basal		Group II Basal plus 100 mg. aureomycin per kg. dry matter consumed		Group III Basal plus 100 mg. chloromycetin per kg. dry matter consumed	
Days on test	9	16	9	16	9	16
Violet red bile agar	2×10^{-8}	2×10^{-9}	4×10^{-9}	—	1×10^{-6}	3×10^{-9}
Bacto-E C medium	10^{-10}	10^{-10}	10^{-10}	10^{-10}	10^{-6}	10^{-10}
Lactalysate broth	10^{-11}	—	10^{-11}	—	10^{-9}	—

Discussion

It is postulated that antibiotics in some manner favorably influence the intestinal flora, possibly by inhibiting undesirable bacteria that have a retarding effect on the growth rate or by increasing the number of bacteria which may synthesize unknown factors required by the pig or known factors present in suboptimal amounts. It has been suggested by Sieburth *et al.* (1951) that antibiotics decrease the number of *Clostridia perfringens,* thus preventing enterotoxemia. However, in the experiments reported herein, there was no evidence of *Clostridia*-type organisms being present in the feces of baby pigs which were removed from their dams at two days of age and reared under laboratory conditions in wire screen bottom cages.

That different antibiotics may act on different microorganisms in the intestinal tract is suggested by the results which show chloromycetin to have had a retarding effect on *E. coli* counts. By the end of 16 days the coli count had returned to normal in the feces of the chloromycetin-fed pigs, perhaps due to an increase in resistant strains; however, the growth effect of the antibiotic remained.

It appears that the antibiotics used, as well as the basal diet, species of animal, and environmental conditions imposed, affects the results obtained from antibiotic feeding.

Summary

Both aureomycin and chloromycetin stimulated the feed consumption and rate of gain of baby pigs fed an alpha-protein "synthetic milk" diet. Chloromycetin reduced the number of coliform bacteria present in the feces during the first nine days of the experiment. This was only a temporary reduction, as by the sixteenth day no difference was apparent. Aureomycin had no effect on the fecal bacteria studied in these tests.

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THE EFFECT OF TERRAMYCIN ON THE GROWTH OF PIGS FED DIFFERENT LEVELS OF PROTEIN[1]

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Michigan Agricultural Experiment Station

THE positive effect upon growth rate resulting from the addition of antibiotics to most swine rations is now a well-accepted fact. However, the relationship between antibiotics and the utilization of other nutrients, particularly protein, is not clear. Cunha *et al.* (1950) working with young growing pigs fed rations varying in protein content from 12.2 percent to 19.6 percent with and without an APF concentrate (Lederle). In each case the addition of APF increased the rate of gain, and the 17.9 percent protein ration with APF was equal to the 19.6 percent protein ration with APF. These same workers found that pigs on a 19.6 percent protein ration without the addition of supplementary B vitamins and without APF did not gain as well as pigs on a 12.2 percent protein ration with B vitamins and APF. In this connection, Sure and Romans (1948) found that increasing the vitamin B complex content of low protein rations fed to rats resulted in marked increases in growth and great improvement in food utilization.

Cunha *et al.* (1949) have also reported a sparing relationship between APF (Lederle's) and the methionine needs of the young pig. Bloss *et al.* (1950) obtained no positive effect from adding aureomycin to tryptophane low rations fed to pigs.

Burnside *et al.* (1951) fed high, intermediate and low protein all vegetable type rations to pigs with and without vitamin B_{12} and aureomycin. Aureomycin improved all rations and the intermediate protein ration with aureomycin was equal to the high protein ration with aureomycin. However, when aureomycin and B_{12} were omitted the higher protein rations were superior to the ones containing less protein. There was no supplementation with additional B vitamins.

Warden *et al.* (1951) have compared various soybean oil meals with meat and bone scraps at two levels of protein (18 and 16 percent) and have reported no significant difference in gains of pigs between the two levels of crude protein fed.

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[2] Departments of Animal Husbandry, Agricultural Chemistry and Animal Pathology, Michigan State College, East Lansing, Michigan. This work was supported in part by a grant from Central Soya Co., Inc., Fort Wayne, Indiana. The authors are also indebted to Charles Pfizer Co., Inc., Brooklyn, N. Y. for the terramycin, and to Merck & Co., Inc., Rahway, N. J. for the B vitamins used in this study.

Work at the Iowa station by Catron *et al.* (1951) indicates that a 16 percent protein level is superior to higher protein levels and that aureomycin has a sparing effect on protein requirements of pigs. Comparisons were made with rations varying from 14 to 20 percent crude protein.

With semi-purified diets in which natural protein sources were used, excellent growth has been obtained with young pigs on rations as low as 11 percent protein (Geurin *et al.*, 1951; Warden *et al.*, 1951).

Experimental

Eighty cross-bred pigs averaging 6–7 weeks in age were used to form eight lots of experimental pigs. The average weight of the eighty

TABLE 1. COMPOSITION OF EXPERIMENTAL RATIONS[1]

Ingredients	Ration	
	Low Protein	High Protein
	Percent	Percent
Corn	80.0	73.0
Soybean oil meal (solvent)	15.0	22.0
Fish Solubles (Menhaden)	2.0	2.0
Limestone	1.4	1.4
Dicalcium phosphate (Pura Phos)	1.0	1.0
Iodized salt	0.5	0.5
Trace mineral premix (CCC)	0.1	0.1
Crude Protein	14.94	18.06

[1] Both rations were supplemented with niacin, riboflavin, pantothenic acid and B_{12} in amounts of 6, 2, 4, and 12.5 micrograms per pound of feed respectively. Ample amounts of vitamins A and D were added to both rations.

pigs was 25±.19 pounds. The pigs were sprayed and wormed at the start of the experiment and were self-fed mixed rations in paved lots.

There were two lots on each treatment and the four treatments consisted of two levels of protein (18 and 15 percent) with and without the antibiotic terramycin (5 mg. per lb. of ration). When the pigs reached an average weight of 100 pounds the protein level was dropped to 15 and 12 percent respectively. B vitamins including B_{12} and vitamins A and D were added to each ration. The composition of the experimental rations is shown in table 1.

Results and Discussion

The results are summarized in table 2. Terramycin had a highly significant effect on rate of gain of the pigs on both levels of protein.

On the higher protein ration the pigs receiving terramycin gained 21 percent faster and required 7.5 percent less feed per unit of gain that those which received no antibiotics. When terramycin was added to the lower protein ration there was a 25 percent increase in rate of gain and a 5 percent improvement in feed efficiency. Practically all the response due to the antibiotic was built up during the period from 25 to 100 pounds, since there was only a 5 percent advantage in favor of the antibiotic fed pigs from 100 to 200 pounds. During the early growth period the terramycin fed pigs gained 37 percent faster than

TABLE 2. THE EFFECT OF TERRAMYCIN ON THE GROWTH OF PIGS FED DIFFERENT LEVELS OF PROTEIN[1]

Protein level up to 100 lbs., % Protein level after 100 lbs., %	18 15		15 12	
Terramycin added, mg./lb. ration	0	5	0	5
No. of pigs	20	20	19[2]	20
No. of days on feed	126	105	122	105
Av. initial wt. and std. error, lbs.	25±.67	25±.63	25±.62	25±.64
Average final wt., lbs.	195	196	189	198
Av. daily gain and std. error, lbs.	1.35±.06	1.63±.04**	1.32±.06	1.65±.04**
Average daily feed cons., lbs.	4.90	5.46	4.80	5.70
Average feed per lb. gain, lbs.	3.62	3.35	3.63	3.45

[1] Two lots of ten pigs each started on each treatment.
[2] One pig removed because of hernia.
** $P<.01$.

those not receiving the antibiotic. The effect of protein level on rate of gain was negligible. There was a remarkable similarity between the growth rates of the pigs on the high and low level protein rations. This similarity held for both early growth (25 to 100 lbs.) and the late growth and fattening (100 to 200 lbs.) periods. The response in growth of the pigs when terramycin was added to the ration was essentially the same at both protein levels.

In view of the fact that most recent feeding standards (National Research Council 1950) recommend a minimum of 18 percent protein for 50 pound pigs it is significant to note that in this experiment 25 pound pigs receiving only 15 percent protein (dropped to 12 percent at 100 pounds) performed just as well as pigs receiving 18 percent protein (dropped to 15 percent at 100 pounds). A possible explanation for the excellent performance of the low protein pigs may be the effect of added B vitamins. Other work at this station (Luecke *et al.* 1952) indicates a much greater percentage response from added pantothenic acid to low protein rations than to high protein ones. This result is in accord with that reported earlier by Sure and Romans (1948) with rats.

Summary

Eighty 25 pound pigs were used to study the effect of terramycin on the growth of pigs fed different levels of protein.

Terramycin at 5 mg. per pound of total ration had a highly significant effect on rate of gain and also improved efficiency of gain. It did not seem to affect the requirement of the pigs for protein.

The pigs receiving the 15 percent protein ration (reduced to 12 percent at 100 pounds) did just as well as the pigs receiving the 18 percent protein ration (reduced to 15 percent at 100 pounds).

It is suggested that our present standards for protein for pigs may be higher than they need to be, and that the high level of B vitamins used in this study may be related to an increased efficiency of protein utilization.

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METHIONINE AS A SUPPLEMENT TO SOYBEAN OIL MEAL FOR WEANLING PIGS[1]

L. V. CURTIN, J. K. LOOSLI, J. P. WILLMAN and H. H. WILLIAMS

Cornell University[2]

THERE has been considerable interest in recent years in the supplementary value of methionine to soybean oil meal in rations for poultry and growing pigs. It has been shown that rations containing a high percentage of soybean oil meal were deficient in methionine for chicks (Almquist *et al.* 1942; Gerry *et al.* 1948). When the diet contains 20 percent protein, the requirement of chicks for methionine plus cystine is reported to be 0.8 percent by Grau and Kamei (1950) and 0.9 percent by Almquist (1947). This is considerably higher than the values that have been reported for swine. Bell *et al.* (1950) have suggested that on a ration containing 10 percent protein, the methionine requirement for growing pigs is between 0.07 and 0.27 percent of the diet. More recently Shelton *et al.* (1951) reported excellent growth of pigs on a 21 percent protein ration supplemented to contain 0.6 percent methionine. They also demonstrated that 0.3 percent of L-cystine could replace one-half of the methionine.

The need for methionine supplementation of soybean oil meal and corn-soybean oil meal rations for swine has been investigated in recent years by several groups of workers with conflicting results. Bell *et al.* (1950) obtained a growth response when methionine was added to a 10 percent protein soybean oil meal ration for growing swine. Dyer *et al.* (1949) observed a growth response with growing swine when methionine was added to a corn-soybean oil meal ration containing 20 percent protein. Added methionine was of no value in the presence of supplemental choline. In contrast, studies of Ferrin (1946), in which approximately 17.5 percent protein rations were fed, and Robison (1951), in which approximately 15 percent protein rations were fed, showed that synthetic methionine was of no benefit when added to rations of yellow corn, soybean oil meal, ground alfalfa and minerals for growing-fattening pigs.

The two studies reported herein were designed to investigate further the need for methionine supplementation of solvent extracted soybean oil meal for growing-fattening pigs and to obtain additional data on the methionine requirement of weanling pigs.

[1] These studies were supported in part by a grant to Cornell University from the Herman Frasch Foundation.

[2] Departments of Animal Husbandry, Biochemistry and Nutrition.

Procedure

The basal rations fed are shown in table 1. No vitamin B_{12} was added to the ration since it has been shown that vitamin B_{12} has a sparing effect on the methionine requirement for swine (Cunha *et al.* 1949). Likewise, no supplemental choline was added for the same reason (Dyer *et al.* 1949). Ration A was fed in the first trial, an individual feeding test with balance studies, and differed from ration B

TABLE 1. COMPOSITION OF BASAL RATIONS

Constituents	A	B
Soybean oil meal, extracted	47.1	47.1
Glucose	25.0	25.0
Lard	5.6	3.6
Ruffex[1]	7.5	1.5
Minerals[2]	4.0	4.0
Vitamins[3]	+	+
Starch[4]	10.3	18.3

1 A commercial rice hull preparation by Fisher Scientific Company.

2 $CaHPO_4 \cdot 2H_2O$, 56.2%; $CaCO_3$, 21.6%; NaCl, 21.6%; $FeSO_4 \cdot 7H_2O$, 0.48%; KI, 0.03%; $MnSO_4 \cdot 5H_2O$, 0.06%; $CuSO_4 \cdot 5H_2O$, 0.03%; $CoCl_2$, 0.015%; $ZnCl_2$, 0.015%.

3 The following vitamins were added to each 100 pounds of basal mixture: Thiamine hydrochloride, 95 mg.; riboflavin, 140 mg.; niacin, 468 mg.; calcium pantothenate, 670 mg.; pyridoxine hydrochloride, 110 mg.; folic acid, 27 mg.; vitamin A, 100,000 I.U. and vitamin D, 10,000 I.U., both supplied by a concentrated fish liver oil; mixed tocopherols, 9 mg.

4 In the formation of experimental rations, DL-methionine, starch, and glycine were added to bring the total ingredients to 100 percent.

in that ration A contained 6 percent more fiber in the form of ruffex and 2 percent more lard. The higher level of ruffex produced firmer feces which enabled more accurate collections to be made in the balance studies. For the second trial (ration B), a group feeding test, the ruffex was decreased since no balance studies were made. The level of lard was also decreased to facilitate mixing of the ration. The changes had no apparent effect on palatability or growth responses, although the pigs in the group feeding test receiving ration B tended to have looser feces throughout the test.

The solvent extracted soybean oil meal fed in both trials was purchased on the local market and found on analysis to contain the following percentages: moisture 10.7, protein 46.1, fat 0.9, crude fiber 5.2, ash 5.0, methionine 0.66,[3] cystine 0.80,[3] and choline 0.32.

Three levels of DL-methionine were added to the basal mixtures to give four experimental rations of varying methionine content. Glycine was then added to equalize the protein ($N \times 6.25$) content of each ration at 22 percent.

3 Details of the methods will be published later.

In the individual feeding test, growth rates, feed efficiency and nitrogen balances were used as measures of the responses of weanling pigs to the different levels of methionine. The pigs were fed twice daily during the six weeks feeding period. Daily feed allowances were periodically equalized at approximately 5 percent of body weight. For

TABLE 2. AVERAGE DATA FOR PIGS RECEIVING VARIOUS LEVELS OF DL-METHIONINE

	Supplemental Level of DL-Methionine (%)			
	0.0	0.1	0.3	0.5
Total DL-Methionine, percent of diet	0.31	0.41	0.61	0.81
	Trial A—Individual Feeding Test			
Number of pigs	5	5	5	5
Initial weight (lbs.)	25	25	25	25
Daily feed intake (% of body weight)	4.37	4.29	4.27	4.36
Daily gain (lb.)	0.79	0.87	0.87	0.78
Feed per pound gain (lbs.)	2.3	2.1	2.1	2.3
Apparent digestibility of nitrogen (%)[1]	86	86	87	85
Apparently digested nitrogen retained (%)[1]	44	44	49	50
	Trial B—Group Feeding Test			
Number of pigs	7	7	6[2]	7
Initial weight (lbs.)	34	33	34	34
Daily gain (lb.)	0.90	0.92	0.93	1.02
Feed per pound gain (lbs.)	2.9	2.7	3.0	2.6

[1] Duplicate determinations on two barrows receiving each ration.
[2] One pig removed because of abnormally poor gains.

nitrogen balances, fecal and urine collections were made over a five-day period following a preliminary period of a minimum of ten days. Adjustable cylindrical cages (Bell, 1948) were used during collections.

The pigs in the group feeding test were fed ration B supplemented with the same levels of DL-methionine as in the individual feeding test. The pigs were fed *ad libitum*. Growth rates and feed utilization were used as measures of the responses to the different levels of methionine in this experiment.

Results

The results of the two tests are given in table 2. In trial A, the individual feeding test, there was a slight growth response when the

ration was supplemented with either 0.1 percent or 0.3 percent DL-methionine; however, 0.5 percent DL-methionine in the ration produced gains similar to the basal ration. None of the differences was statistically significant. Feed efficiency paralleled average daily gains in all lots. There was some increase in the percentage of apparently digested nitrogen retained in the groups receiving the two highest levels of methionine.

In trial B, the group feeding test, the pigs receiving 0.5 percent added DL-methionine made slightly faster gains with less feed per 100 pounds of gain than those receiving the unsupplemented basal ration. There were no differences in the gains of the other three lots.

The average daily gains of all the pigs in the two trials for each of the four levels of methionine were 0.86, 0.90, 0.90, and 0.92 pounds. None of the differences was statistically significant. The average amounts of feed per 100 pounds gain in the two trials for each of the four levels of methionine were 269, 246, 262, and 252 pounds.

From these data, it is clear that for the solvent extracted soybean oil meal used, there is no significant advantage in adding supplemental DL-methionine to the 22 percent protein ration for growing-fattening pigs. Moreover, the data indicate that the DL-methionine requirement for growing-fattening pigs does not exceed 0.31 percent when the ration contains 0.38 percent cystine and 22 percent protein. The reason that methionine supplementation of soybean oil meal may be needed for maximum growth of chicks and not for weanling pigs is apparently a species difference in the requirement for the sulfur containing amino acids. The requirement of methionine plus cystine for swine, 0.6 percent as reported by Shelton *et al.* (1951) or not more than 0.69 percent as reported herein, is considerably lower than that required by chicks (Almquist, 1947; Grau and Kamei, 1950). When expressed as percentages of the protein, the value for swine is at least 36 percent lower than the requirement for poultry.

In the experiments reported in this paper and in the experiment of Ferrin (1946) in which methionine did not improve a corn-soybean oil meal ration for swine, solvent extracted soybean oil meal was used, whereas expeller soybean oil meal was used by Bell *et al.* (1950) and Dyer *et al.* (1948) in experiments which responded favorably to methionine supplementations. Dyer *et al.* (1950) and Robison (1951) have reported that solvent extracted soybean oil meal produced a faster rate of gain for swine than expeller soybean oil meal. These differences may be due to the variation in the amount of heating

used in the processing of the two meals since Reisen *et al.* (1947), confirming earlier studies, have reported a decrease in the availablity of methionine in soybean oil meal caused by excessive heat treatmnt.

Summary

Two experiments were conducted to study the effect of adding DL-methionine to a basal ration containing 22 percent protein from solvent extracted soybean oil meal, without supplemental choline or vitamin B_{12}, for weanling pigs. Growth, feed efficiency, and nitrogen balance data indicate that there was no significant advantage in adding supplemental DL-methionine to the sample of solvent extracted soybean oil meal used. Since soybean oil meal is lower in methionine than the other common swine feeds, including corn, the data suggest no practical need for methionine supplementation in swine rations containing good quality solvent extracted soybean oil meal as the only high protein feed.

The data presented also show that the methionine requirement for growing-fattening pigs does not exceed 0.31 percent when the ration contains 0.38 percent cystine and 22 percent protein. This requirement of methionine plus cystine for swine is considerably lower than that required for chicks.

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PENICILLIN, LYSINE, METHIONINE AND FISH SOLUBLES SUPPLEMENT A CORN-DEGOSSYPOLIZED COTTONSEED MEAL RATION FOR WEANLING PIGS

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COTTONSEED meal is second only to soybean oil meal among the protein concentrates in volume produced. However, before this product can serve as a satisfactory major source of protein for swine it is necessary not only to detoxify it efficiently but also to determine and correct its nutritional deficiencies.

The use of commercial cottonsead meal as a protein supplement for pigs in the past has been limited by its toxicity rather than its protein quality. Cottonseed meal toxicity has been commonly measured in terms of gossypol content. Efforts to improve cottonseed meal as a protein supplement, however, by lowering its gossypol content have met with only limited success.

Dyer *et al.* (1951) fed pigs a corn-cottonseed meal ration in which the free gossypol content of the cottonseed meal was 0.03 percent or less. Severe dermatitis occurred when this ration was fed, indicative of a "toxicity" or a deficiency of the ration. It is unlikely that the level of gossypol in the diet (<.01 percent) would have produced such ill effects.

Boatner *et al.* (1948) suggested the presence of toxic components of cottonseed meal, other than gossypol, that have a deleterious effect on chick growth. Ambrose and Robbins (1951) using the rat as the test animal also concluded that a toxic constituent, other than gossypol, is present in the glands.

On the other hand, data presented by Lillie and Bird (1950) indicate that pure gossypol was as toxic as the pigment gland containing an equivalent amount of gossypol. This would suggest that the deleterious effects experienced from the use of degossypolized cottonseed meal may be the result of a nutritional deficiency rather than a toxicity.

Hamilton (1950) stated that lysine is the limiting amino acid in cottonseed meal. Richardson and Blaylock (1950) working with poults also reported that the lysine content was too low in a ration in which cottonseed meal was used as the protein supplement.

[1] Acknowledgement is made to J. L. Carmon, instructor, department of animal husbandry, for his assistance in the statistical analysis of the results of these experiments.

Brinegar *et al.* (1950) reported that the l-lysine requirement of the pig when fed a 22 percent protein diet was 1.1 percent of the ration. Approximately twice this level of dl-lysine is required since the pig uses only the l-isomer. Since cottonseed meal contains approximately 2.25 percent lysine (Lyman *et al.* 1947) it appears likely that suboptimal performance of pigs will result when an unsupplemented corn-cottonseed meal ration is fed.

Methionine has been shown to supplement a corn-soybean meal ration for pigs (Dyer *et al.* 1949). Since the average methionine composition of cottonseed meal is essentially the same as in soybean oil meal it appears probable that methionine would increase the growth rate and improve feed efficiency when fed with a corn-cottonseed meal ration.

The methionine content, by calculation, of a 20 percent crude protein corn-cottonseed meal ration is approximately 0.36 percent or about one-half the pigs' requirement.

Fish solubles were reported by Noland (1951) and others to have improved a corn-cottonseed meal ration for pigs. Richardson and Blaylock (1950) reported a variable response of poults when fish solubles were added to a corn-cottonseed meal ration. In some trials a marked response occurred while in others the fish solubles had little or no effect on performance.

Bridges *et al.* (1951), in two tests with pigs fed a corn-cottonseed meal ration plus penicillin reported an improved growth rate and feed efficiency over pigs fed a comparable ration without penicillin. Luecke *et al.* (1950) also stated that penicillin improved the growth rate of pigs fed a corn-soybean oil meal ration.

Two experiments, in which cottonseed meal of low gossypol content was used, were conducted to (1) study the value of the degossypolized product as a protein supplement for pigs and (2) to determine the effect of penicillin, lysine, methionine and fish solubles on the growth rate of weanling pigs when fed with a corn-degossypolized cottonseed meal ration fortified with minerals and vitamins.

Experimental Procedure

Experiment 1

Weanling Duroc, Poland China and Duroc × Poland China pigs that had been in drylot since birth were divided into six comparable groups of eight pigs each. All groups were self-fed a 20 percent crude protein ration in drylot to an average final weight of 100 pounds. The various rations fed are presented in table 1. Water was available for *ad libitum* consumption.

TABLE 1. RESPONSE OF PIGS TO VARIOUS SUPPLEMENTS WHEN ADDED TO A CORN-COTTONSEED MEAL RATION IN DRYLOT (1951 WINTER)[1]

	Lot					
	1	2	3	4	5	6
	Corn 63%, cottonseed meal, 35%,[2] Minerals, 2%,[3] Vitamins[4]	1 + 0.02 % dl-lysine	1 + 3% fish solubles[5]	Corn, 63.0% soybean meal, 17.5%, cottonseed meal, 17.5% minerals, 2.0% Vitamins	Corn, 63.0% soybean meal, 8.7%, cottonseed meal, 26.3%, minerals, 2.0% Vitamins	4 + penicillin[6]
No. pigs on test	8	8	8	8	8	8
Av. initial weight, lb.	28.1	29.7	31.5	30.0	29.9	30.1
Av. final weight, lb.	70.1	85.8	92.2	99.6	100.0	100.5
Av. daily gain, lb.	0.63	0.80	0.86	1.16**	0.99**	1.24**
and S. E.	±0.10	±0.09	±0.08	±0.08	±0.07	±0.08
Av. daily feed/pig, lb.	3.31	3.51	4.15	3.50	3.34	3.69
Feed/100 lbs. gain, lb.	501.	436.	484.	302.	338.	299.

** Gained faster (P≦0.01) than pigs fed the basal ration. Also groups 3 and 6 gained at a significantly faster rate (P≦0.01) than group 2; group 3 significantly faster (P≦0.01) than 5; group 6 significantly faster (P≦0.05 and 0.01) than 4 and 5 respectively. After removal of differences due to variations in initial weight, by analysis of covariance, the difference between treatments remained significant.

[1] The authors are grateful to: Mr. O. H. Alderks and Mr. J. T. Raper, The Buckeye Cotton Oil Co., Ivorydale, Ohio, for supplying the cottonseed and soybean oil meals and fish solubles; Dr. M. A. Schooley, Merck and Co., Inc., Rahway, New Jersey, for the riboflavin, niacinamide, calcium pantothenate, choline chloride, vitamin B_{12} supplement, and penicillin; Dr. H. H. Havner, The Borden Co., New York, New York, for the vitamin A and D supplement; Dr. J. Waddell, E. I. du Pont de Nemours and Co., New Brunswick, New Jersey, for the dl-lysine HCl. Acknowledgement is made to Mr. D. A. Hart, Herdsman, for his assistance in conducting this test.

[2] Less than .03 percent gossypol.

[3] Composed of 2 parts Deflorophos, 2 parts ground limestone and 1 part salt.

[4] Vitamin additions per pound of ration were: 500 IU vitamin A, 90 IU vitamin D, 1.5 mg. riboflavin, 5 mg. niacinamide, 6 mg. calcium pantothenate, 250 mg. choline chloride and 15 mcg. vitamin B_{12} activity.

[5] Contained 51.5 percent dry matter, 35 percent crude protein and 5.94 percent ether extract.

[6] Procaine penicillin G (1,000,000 units per gram).

Experiment 2

Five comparable groups of ten weanling Duroc and Poland China pigs each were self-fed as described above. Rations fed are presented in table 2. Data in both experiments were analyzed statistically by analysis of variance.

Results and Discussion

Experiment 1

The eight pigs receiving the basal ration became dermatitic within 56 days after being placed on test. Pigs receiving this ration gained at only 0.63±.10 pound per day. These eight pigs were removed from test (70 pounds liveweight) because of severe dematitis. The addition of penicillin to the above diet subsequent to the removal from test alleviated the dermatitis within four weeks and increased the growth rate.

An average daily gain of 0.80±0.09 pound was produced when 0.2 percent dl-lysine was added to the basal ration (group 2). This rate of gain was suboptimal probably because the total lysine content of the basal ration, by calculation, was only 0.8 percent. It is now known that this level of lysine is suboptimal for the pig. Five of these pigs developed dermatitis. This condition was arrested and the growth rate and feed efficiency were improved by replacing one-half the cottonseed meal with solvent soybean oil meal in post-experimental treatment.

Supplementation of the basal ration with 3 percent condensed fish solubles (group 3) supported a satisfactory growth rate (1.05±.08 pounds average daily gain) up to an average of 85 pounds liveweight. At this weight, growth was arrested and all pigs became dermatitic. The condition of these pigs deteriorated during the subsequent 21 day period. Consequently, they were removed from test. This poor performance might be partially explained in that the fish solubles added both choline and vitamin B_{12} to a ration already having an "excess" of these vitamins. The last three weeks, during which no weight increase occurred, resulted in a very poor feed efficiency as well as lowering the average daily gain.

When the protein supplement contained equal parts of solvent soybean oil meal and "degossypolized" cottonseed meal the rate of gain and appearance of pigs were satisfactory (group 4). Neither dermatitis nor depigmentation of the haircoats were observed. It was not determined whether the reduced level of cottonseed meal *per se* or an improvement

TABLE 2. SUPPLEMENTING A CORN-DEGOSSYPOLIZED COTTONSEED MEAL RATION WITH FISH SOLUBLES, LYSINE AND METHIONINE FOR PIGS (1951 SUMMER)[1]

	Lot Numbers and Treatment				
	1	2	3	4	5
	63% corn, 35% cottonseed meal, 2% minerals and Vitamins[2]	62% corn, 33% cottonseed meal, 3% fish solubles, 2% minerals and Vitamins[2]	Same as 1 + 0.4% dl-lysine	Same as 1 + 0.5% dl-methionine + 0.4% dl-lysine	64% corn, 34% soybean oil meal, 2% minerals and Vitamins[2]
No. pigs allotted	10	10	10	10	10
No. pigs that finished test	7	10	10	10	9
Av. initial weigth, lb.	36.0	36.0	36.5	36.1	36.2
Av. final weight, lb.	106.6	110.3	100.1	98.5	97.3
Av. daily gain, lb.	0.79	1.26**	1.11	1.19*	1.27**
and Standard error	±0.14	±0.07	±0.09	±0.06	±0.04
Av. feed/pig/day, lb.	3.82	4.05	4.25	4.34	3.63
Feed/100 lbs. gain, lb.	415.	321.	375.	366.	287.

*, ** Significantly faster (5%; 1% levels of probability respectively) rate of gain than produced by the basal ration. Also, pigs in lot 5 gained significantly ($P=0.05$) faster than those in lot 3.

[2] Vitamin additions per pound of ration were: 500 IU vitamin A, 100 IU vitamin D; .5 mg. B_1; 1.0 mg. B_2; 7.5 mcg. B_{12}; 2.0 mg. niacin; 5.0 mg. pantothenic acid and 300 mg. choline chloride.

[1] The cottonseed meal, fish solubles, soybean oil meal and a portion of the lysine and methionine were supplied through the courtesy of Dr. Kenneth Kuiken of the Buckeye Cotton Oil Co., Ivorydale, Ohio Vitamins B_1, B_2, niacinamide, calcium pantothenate and B_{12} were generously contributed by Drs. M. A. Schooley and L. Michaud, Merck and Co., Inc., Rahway, New Jersey. Vitamins A, D and choline chloride were contributed by the Dawe's Manufacturing Co., Chicago, Illinois, through the courtesy of Mr. Vernon Dawe. A portion of the dl-lysine and dl-methionine were furnished by Dr. J. Waddell, du Pont de Nemours and Co., New Brunswick, New Jersey.

in the balance of the ration accounted for the absence of dermatitis in this group.

Pigs in group 5 exhibited a slower rate of gain, dermatitis and a poor feed efficiency when the cottonseed meal was increased to constitute 75 percent of the protein supplement. This level of cottonseed meal contained enough of the factor(s) responsible for, or created an inbalance sufficient to cause, dermatitis.

Pigs receiving a ration in which cottonseed meal was used as three-fourths of the protein supplement and soybean oil meal the remaining one-fourth (group 6) gained significantly faster when 2.27 mg. of penicillin per pound of diet was added than when this ration was fed without penicillin.

The mode of action of penicillin in stimulating growth is a question of interest. It was observed that the number of bacteria in the intestinal tract was significantly increased when penicillin was fed. It is possible that this change in the microflora promoted a synthesis of growth factor(s) needed by the pig.

A more rapid rate of gain probably would have occurred in all groups had vitamin B_{12} been fed at a level lower than 15 mcg. per pound of ration. Observations corroborating this supposition were made by Dyer and Krider (1950) with pigs and Schaefer *et al.* (1949) with chicks where it was shown that "high" levels of B_{12} and methyl group donors fed concurrently reduced performance. Depigmentation of the hair coat of pigs in this experiment was less severe than those in an earlier experiment reported by Dyer *et al.* (1951) in which pigs received a similar ration.

Experiment 2

Pigs fed the basal ration (63 percent corn, 35 percent cottonseed meal plus minerals and vitamins) made unsatisfactory gains (.79 pounds per day). Three of the ten pigs in this group did not finish the test. Gains were variable in this group as shown by a higher standard error of the mean (± 0.14 pound). Dermatitis and haircoat depigmentation occurred in this group although neither condition affected all the pigs. Performance in this group was superior to that of the basal group in experiment 1. Perhaps no significance should be attached to this since different seasons were involved and too, pigs in experment 2 averaged approximately 8 pounds heavier when placed on test.

The addition of 3 percent fish solubles to the basal ration (group 2) very significantly (1 percent level) increased the average daily gain.

These results are somewhat better than obtained in experiment 1 when fish solubles were fed. The discrepancies might be reconciled in that either or both the cottonseed meal and fish solubles used in this test varied markedly in quality from those used in experiment 1. It is also possible that the reduction in vitamin B_{12} from 15 mcg. in experiment 1 to 7.5 mcg. per pound of ration in experiment 2 resulted in improved performance. Biological, seasonal and initial weight differences might account for some of the difference in rates of gain. Neither dermatitis nor depigmentation of the haircoats were observed in this group of pigs.

The basal ration supplemented with 0.4 percent dl-lysine produced an average daily gain of 1.11 ± 0.09 pounds. In experiment 1 when 0.2 percent lysine was fed the average daily gain was 0.80 ± 0.09 pound. The feed required per 100 pounds gain was 375 and 436 pounds when 0.4 and 0.2 percent lysine respectively were fed. From these results it appears that the ration was suboptimal in its lysine content when 0.2 percent was added. Although results were "satisfactory" when 0.4 percent dl-lysine was added to the diet it is probable that still better results would have been obtained had the ration contained a higher level of lysine.

An average daily gain of $1.19 \pm .06$ pound was produced when 0.5 percent dl-methionine was added to the corn-cottonseed meal ration containing lysine (group 4). More uniformity was also observed in this group than in group 3. It was not determined whether the methionine *per se* increased the performance or whether it was merely supplying labile methyl groups. The average daily gain in this group was significantly faster (5 percent level) than that made by the basal group.

Methionine has been shown many times to increase the growth rate of pigs fed a corn-expeller soybean oil meal ration. Since the methionine content of cottonseed and soybean oil meals are essentially the same, (Lyman *et al.* 1947) one might expect to obtain a response from methionine when added to a corn-cottonseed meal ration.

A corn-solvent soybean oil meal ration was used as a positive control. Excellent growth rates ($1.27 \pm .04$ pounds), feed efficiency and uniformity were produced by this ration. This rate of gain was highly significantly faster (1 percent level) than that made by pigs fed the basal ration and was slightly superior to the average daily gain of 1.26 ± 0.07 pounds produced by the corn-cottonseed meal ration plus fish solubles (group 2). Only 287 pounds of feed were required to produce 100 pounds gain as compared to 321 pounds in group 2.

Summary and Conclusions

The corn-"degossypolized" cottonseed meal ration fortified with minerals, vitamin A, D, riboflavin, niacin, pantothenic acid, choline and B_{12} although producing no typical symptoms of gossypol toxicity, did not support optimum performance. Neither 0.2 percent dl-lysine nor 3 percent condensed fish solubles significantly increased this rate of gain in one test. In a second test 3 percent fish solubles resulted in a highly significant increased growth rate. The growth rate of pigs fed 0.4 percent dl-lysine was 0.32 pounds per day faster than that made by pigs fed a comparable ration without lysine. The further addition of 0.5 percent dl-methionine to the corn-cottonseed meal ration plus lysine resulted in a significantly faster average daily gain than that made by pigs in group 1. When one-half the cottonseed meal was replaced by solvent soybean oil meal the rate of gain was significantly increased. When only one-fourth the cottonseed meal was replaced by soybean oil meal, gains were not as satisfactory as when one-half the cottonseed meal was replaced. The further addition of penicillin significantly improved the rate of gain and appearance of pigs.

Although degossypolized cottonseed meal did not produce an optimum growth rate when fed as the only protein supplement to pigs, it is significant that the toxicity has been reduced to an extent that larger amounts can be incorporated in the ration. Consequently, its value as a protein supplement can be more readily determined.

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EFFECTS OF PENICILLIN AND STREPTOMYCIN ON THE GROWTH RATE AND BACTERIAL COUNT IN THE FECES OF PIGS

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VARIOUS antibiotics have been shown to stimulate growth rate in weanling pigs. Luecke (1950) reported that streptomycin had a growth promoting effect in the pig. Dyer *et al* (1950) likewise reported an increase in growth rate of weanling pigs fed a corn-cottonseed meal ration when streptomycin was added. Luecke *et al.* (1950) reported a significant increase in growth rate of pigs fed a corn-soybean oil meal ration with penicillin at levels of 1 and 5 mg. per pound of feed. However, streptomycin did not result in a significant increase in growth rate.

Nesheim (1950), working with baby pigs fed a "synthetic" milk diet, reported a highly significant increase in average daily gain and efficiency of feed utilization of pigs when 500 mg. streptomycin/kg. dry matter was fed. Pigs were pair-fed with equal feed intake for 49 days.

Bird *et al.* (1950), Groschke (1950), McGinnis (1950) and Stokstad (1950) reported increased growth rate in chicken and turkey poults when streptomycin was included in the ration.

Wahlstrom (1950) reported no significant increase in weight of baby pigs on a "synthetic" milk diet after eight weeks of feeding penicillin at the rate of 100 mg. per kg. of dry matter. Cunha *et al.* (1950) reported no significant increase in growth rate of weanling pigs fed a corn-peanut meal ration with penicillin.

Brown and Luther (1950) reported that the addition of terramycin, streptomycin, penicillin and aureomycin at levels as low as three parts per million gave a significant increase in growth rate of pigs over a "balanced" all vegetable ration containing vitamin B_{12}, and showed a comparable superiority over practical rations containing animal protein. The animals receiving antibiotics showed increased growth

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responses of about 40 percent at four weeks of age. However, this rate of growth was not maintained throughout the test.

Reports generally indicate that the extent of the stimulus varies depending on the antibiotic used, the frequency and amount fed, the method of administration and the type of ration used. In addition the level of the various B vitamins fed are believed to influence the effect of antibiotics on growth rate.

Effects of combining penicillin and streptomycin in the diet are not known.

Experimental Procedure

The objects of the two growth tests were:

1. To study the effect of penicillin, streptomycin and a combination of penicillin and streptomycin on the growth rate and feed efficiency of pigs fed in drylot from weanling to 100 pounds and from 100 to 200 pounds.
2. To determine if a significant correlation exists between the number of bacteria in the feces and the rate of gain made by the pig.

Five groups of ten Duroc and Duroc × Poland China pigs each were self-fed a 20 percent crude protein corn-degossypolized cottonseed meal ration in drylot to average final weights of 100 pounds. All rations were fortified with minerals and vitamins (table1).

Pigs were allotted according to weight, breed, litter, condition and sex at 56 days of age. All pigs were bedded with wood shavings, confined in concrete-floored pens and given water *ad libitum*. Pigs were weighed at two-week intervals until they approached 100 pounds liveweight, after which they were weighed weekly. Data were analyzed by analysis of variance.

Fecal samples were collected after a five-day pre-treatment period in which all pigs were fed the basal ration. After the pigs were allotted, samples were collected at frequent intervals, refrigerated and counts made within 48 hours. One g. samples were diluted to 1/100, 1/10,000, and 1/1,000,000. Duplicate plates were made on nitrate agar (BBL) from the last two dilutions. Plates were incubated at 32° C for 48 hours at which time colonies on each plate were counted in a Buck Colony counter.

A second experiment was conducted using two comparable groups of eight pigs each fed from 100 to 200 pounds liveweight in drylot.

Group 1 was fed a ration composed of 64 percent corn, 24 percent degossypolized cottonseed meal, 10 percent alfalfa meal and 2 percent mineral mixture. Group 2 received this ration plus 227 mg. of procaine penicillin G per 100 pounds of feed. Other treatments were as described in experiment 1.

TABLE 1. EFFECTS OF PENICILLIN AND STREPTOMYCIN ON THE GROWTH RATE AND BACTERIAL COUNT IN THE FECES OF PIGS

	Lot Numbers and Treatments				
	1	2	3	4	5
	63% corn, 35% degossypolized cottonseed meal,[1] 2% mineral mixture and vitamins[2]	Same as 1 + 227 mg. penicillin/100[3] lbs. feed	Same as 1 + 250 mg. streptomycin/100 lbs. feed	Same as 1 + 227 mg. penicillin and 250 mg. streptomycin/100 lbs. feed	64% corn, 34% solvent soybean oil meal, 2% mineral mixture and vitamins[2]
No. pigs started on test	10	10	10	10	10
Av. initial weight, lb.	36.0	36.2	36.0	36.1	36.2
No. pigs finished[4]	7	9	10	7	9
Av final weight, lb.	106.6	99.4	102.0	109.0	97.3
Av. days on test	68	58	58	58	49
Av. daily gain, lb.	0.79	1.05	1.15*	0.92	1.27**
and standard error	±.14	±.06	±.04	±.17	±.03
Av. daily feed/pig, lb.	3.82	3.68	3.75	3.80	3.63
Feed/100 lb. gain, lb.	415	351	329	343	287
No. of Bact. 1,000,000/g. of feces and	54	271*	59	83	61
standard error	±18.3	±73.3	±12.0	±16.6	±11.0

*, ** Significant (5% and 1% respectively) over the basal group. The rates of gain in lot 5 were significantly faster (1% and 5% level) than that of pigs in lots 2 and 3 respectively.

[1] Contained less than .03 percent free gossypol.

[2] Vitamin additions to the 20 percent crude protein ration were: 500 I.U. Vitamin A; 100 I.U. vitamin D; 0.5 mg. thiamin, 1.0 mg. riboflavin; 2.0 mg. niacinamide; 5.0 mg. calcium pantothenate; 250 mg. choline chloride and 7.5 mcg. B_{12} per pound.

[3] Procaine Penicillin G.

[4] Three pigs removed from lot 1 (2 with dermatitis, 1 died of peritonitis). One pig in lot 2 died of peritonitis. Three pigs removed from lot 4 (2 wth dermatitis, 1 died of necrotic enteritis). One pig from lot 5 died of peritonitis.

Results and Discussion

Pigs in experiment 1 (weanling to 100 pounds liveweight) fed the basal ration performed unsatisfactorily. An average daily gain of only 0.79±0.14 pound was produced by this ration. Feed efficiency was 415 pounds per 100 pounds of gain. Two pigs were dermatitic all pigs exhibited a slight depigmentation of the hair coats.

The addition of 227 mg. of penicillin per 100 pounds of feed resulted in an average daily gain of 1.05±0.06 pounds. However, this rate of gain was not significantly faster than when no penicillin was fed. Pigs

in this group were more uniform, had no severe dermatitis and had a higher feed efficiency than pigs in group 1.

When streptomycin was added to the basal ration (group 3) a significantly faster rate of gain (1.15±0.04 pound) was produced. To 100 pounds liveweight, feed efficiency was 329 pounds per 100 pounds gain. These pigs were kept on the same ration after being weighed off test, and at approximately 150 pounds they became dermatitic and lost weight constantly. This observation was corroborated by Dyer *et al.* (1950) when pigs performed similarly from weaning to 100 pounds and from 100 pounds to 200 pounds liveweight.

The combination of penicillin and streptomycin (group 4) had an adverse effect on the pigs for the first two weeks. Three had to be removed soon after the test began. Rough hair coats and general unthriftiness were observed for the first two weeks. Considering all pigs put on test, the average daily gains were 0.92±0.17 pound. However, of the seven pigs finishing the test the average daily gains were 1.24±0.03 pound.

The rate of gain in group 5 (corn-solvent soybean oil meal ration) was quite satisfactory (1.27±0.03 pound). Feed efficiency was high (287 pounds per 100 pounds gain). Neither dermatitis nor depigmentation of the hair coats occurred. This rate of gain was very significantly faster than in group 1 and was also significantly faster (1 percent and 5 percent) than that made by pigs in groups 2 and 3 respectively.

Pigs fed a corn-cottonseed meal ration from 100–200 pounds liveweight gained 1.58±0.09 pound per day. No external symptoms indicative of a dietary deficiency other than a slight depigmentation of the haircoat were observed.

The addition of penicillin to this ration resulted in an average daily gain of 1.78±0.08 pound. The difference in rates of gain in groups 1 and 2 was not statistically significant. Feed efficiency was slightly higher than in group 1 (table 2).

Tables 1 and 2 show the number of bacteria per gram of wet feces in experiments 1 and 2 respectively. In both experiments the addition of penicillin resulted in a statistically significant increase in total bacterial count. The object of this experiment was not to study the effect of penicillin and streptomycin on the various groups of bacteria, although it was observed that the enterobacteriacae were increased when penicillin was fed. Penicillin is inactive against this group of bacteria.

Bridges and Dyer (1951), in a preliminary test, injected pigs intramuscularly with 18,000 units of penicillin in oil on alternate days. The pigs were fed the basal ration listed in experiment 1. Bacterial counts were significantly increased (1 percent) but no increases in the rates of gain occurred.

Neither the addition of streptomycin nor a combination of penicillin and streptomycin significantly increased the bacterial counts, although

TABLE 2. EFFECTS OF PENICILLIN ON THE GROWTH RATE AND BACTERIAL COUNT IN THE FECES OF HOGS FED A CORN-COTTONSEED MEAL RATION

	Lot Numbers and Treatment	
	1	2
	Corn 64%, cottonseed meal 24%, alfalfa meal 10%, minerals 2%[1]	Same as 1 plus 227 mg. procaine penicillin g/100 lbs. feed
No. pigs on test	8	8
Av. initial wt., lb.	100.4	99.9
Av. final wt., lb.	191.5	196.3
Av. days on test	57.6	54.2
Av. daily gain, lb.	1.58	1.78
and standard error	±0.9	±.08
Av. daily feed/pig, lb.	6.50	7.01
Feed/100 lbs. gain, lb.	407.0	385.0
Bacterial count 1,000,000/g	8.0	247.0**
feces and standard error	±1.9	±42.8

** Significantly greater number than in group 1.

[1] Contained less than .03 percent free gossypol. Mineral mixture contained 2 parts Deflovophos, 2 parts steamed bone meal and 1 part salt.

the bacterial counts in feces of pigs in all groups were higher than from pigs fed the basal ration.

One postulation as to the mode of action of antibiotics in stimulating growth has been that the total bacterial count was lowered, thereby reducing competition between bacteria and the host for nutrients. These results indicate that some mode(s) of action of penicillin and streptomycin other than a reduction in bacterial count is responsible for the increase in growth rate of the pig.

Rates of gain and total bacterial counts were not significantly correlated.

Summary and Conclusions

Two tests are reported in which penicillin and streptomycin, singly and in combination, were added to a corn-cottonseed meal ration.

Pigs fed the rations containing penicillin gained 0.26 pound per pig faster from weaning to 100 pounds and 0.20 pound faster from 100 pounds to market weight than did pigs fed the basal ration. These differences were not statistically significant. However, streptomycin significantly increased the rates of gain over that produced by the basal ration from weaning to 100 pounds.

Bacterial counts in the feces were significantly increased by the addition of penicillin but not by the addition of streptomycin or a combination of streptomycin and penicillin. No significant correlation between total bacterial count and growth rate occurred possibly due to the small number of pigs used.

Feed efficiency was improved by feeding either penicillin, streptomycin or a combination of penicillin and streptomycin.

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THE UTILIZATION OF CERTAIN CELLULOSIC MATERIALS BY SWINE

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A SURVEY of the literature relevant to the degree and manner of utilization of the fibrous portion of feeds by swine reveals that the apparent digestibility of crude fiber may vary widely (Axelsson, 1947; Homb, 1948; Hvidsten and Homb, 1947; Mangold, 1934; Mitchell and Hamilton, 1933; Nordfeldt, 1946b; Phillipson, 1947; Woodman and Evans, 1947a). While it is accepted generally that the "disappearance" of cellulose, constituting the major portion of the crude fiber fraction, during the passage of feedstuffs through the digestive tract is the result of bacterial action (Phillipson, 1947), little has been done toward investigation of the factors which may influence the extent of utilization of cellulosic materials by swine.

The variation in crude fiber digestibility previously reported has not been satisfactorily explained and may be the result of differences in source of fiber employed, level of fiber in the diet, the character of the non-fibrous portion of the ration, the plane of nutrition, and/or the age of the pigs. Direct observations on the degree to which the apparently digested fiber is utilizable by swine are few and contradictory (Breirem *et al.*, 1943; Homb, 1948; Nordfeldt, 1946a; Woodman and Evans, 1947b). A thorough understanding of these matters is essential for accurate evaluation of swine feeding practices, particularly in view of the widespread use of pasturage and of dehydrated roughage for supply of minor nutrient essentials.

The data presented here were obtained in the course of experiments designed to investigate the role of the source of cellulose on its apparent digestibility and on the metabolizable energy of well-balanced rations containing significant amounts of added cellulose. A further purpose of these experiments was to investigate the possible advantages of substituting analyses for cellulose and for lignin in place of the much used and much maligned crude fiber estimation.

[1] The wheat straw pulp was prepared and supplied generously through the courtesy of Dr. E. C. Lathrop, Head of the Agricultural Residues Division, Northern Regional Research Laboratory, Peoria, Illinois.

The supervision of experimental details was handled by W. T. Haines.

The investigations on lignin and cellulose methods were conducted by Lucile Dekker-van Ghyl.

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These experiments were initiated through the stimulus of reports by Woodman and Evans (1947a, 1947b) that growing and fattening pigs could digest 82 percent of the cellulose of wheat straw pulp present in complete rations and that the digested cellulose contributed importantly to the total digestible nutrients of the rations. The wheat straw pulp employed by the English investigators was prepared by treatment of the straw with boiling caustic soda.

TABLE 1. CHEMICAL COMPOSITION OF RATIONS AND SUPPLEMENTS AS FED[1]

Test	Percent of Given Supplement in Ration		Crude Protein, %	Gross Energy cal./gm.	Crude Fiber, %	Cellulose, %	Lignin, %
A	Woodflock	7.3	13.46	3912	10.40	13.54	1.30
	Starch	7.3	13.37	3886	5.01	7.39	1.24
B	Ruffex	7.3	13.39	3963	10.62	12.85	1.86
	Starch	7.3	13.52	3903	5.78	7.37	1.30
C	Wheat Straw Pulp	7.3	13.53	3918	11.02	10.56	1.16
	Starch	7.3	12.75	3882	5.24	5.62	1.18
D	Alfalfa Meal	23.6	15.06	3966	9.78	10.35	....
	Woodflock	6.4	15.16	3968	9.25	13.33	....
E	Wheat Straw	14.4	12.10	3883	9.82	12.11	2.52
	Woodflock	7.1	12.68	3872	9.38	12.40	1.22
F	Oat Hulls	13.6	12.34	3861	8.90	11.90	....
	Woodflock	7.1	12.88	3893	10.20	12.78	....
G	Alfalfa Meal +Sulfathalidine	23.6 1.85	16.03	3924	9.52	10.56	2.81
	Alfalfa Meal	23.6	15.03	3876	9.64	10.42	2.78
Composition of Supplements:							
Woodflock			.13		74.92	92.41	.13
Ruffex			.25		68.35	87.50	4.66
Wheat Straw Pulp					71.67	86.23	.74
Wheat Straw					38.10	46.63	11.18
Oat Hulls			2.59		32.59	48.66	10.50
Alfalfa Meal			17.56		22.00	23.41	9.64

[1] The basal ration was composed of the following ingredients: ground yellow corn 72 percent, meat and bone scraps (50 percent crude protein) 8 percent, expeller soybean oil meal 4 percent, dehydrated alfalfa meal 14 percent, limestone 0.8 percent, steamed bone meal 0.8 percent, and iodized salt 0.4 percent.

Experimental Methods and Materials

Determination of digestibility and metabolizability of rations by swine were made by the procedures commonly employed at this laboratory (Hamilton *et al.*, 1928). The experiments were conducted during the fall and winter of 1948–49 and 1949–50. Six animals were used each season, being Duroc Jersey and Poland China barrows of 125 pounds initial weight. Table 1 shows the order of experimentation and composition of rations and cellulosic supplements. The supplements used in

trials A through C were added to a complete swine ration compounded for general use in the swine herd at this station. In trials D through F, casein, corn oil, bone meal, and starch were used where necessary to balance the pairs of rations for protein and estimated metabolizable energy.

Trials A and B were double reversal trials, while the remaining ones, conducted the following year, were single reversal trials. By the reversal procedure, each animal served as his own control, and the data could be analyzed statistically using the method of paired differences. To rule out the possible effects of previous feeding, both rations were fed in the initial period of each trial, one ration to each of three pigs. In following periods the rations were reversed. All animals in a given period consumed the same amount of feed in equal daily feedings. The amount fed was regulated to permit a gain of about one-half pound daily in body weight of the pigs. There were no feed refusals during any collection periods. All of the pigs appeared to be in good health throughout the experiments.

The usual proximate analyses were performed by the standard procedures of the A.O.A.C. Cellulose was determined by the method of Matrone, Ellis and Maynard (1946) modified as follows:

After extraction with ethanol-benzene, the sample is dried by suction and then suspended in 50 ml. of distilled water and brought to a boil. The solution is then cooled to 30° C. and 3 ml. of mylase-buffer solution are added. Digestion is continued at 30° C. for one hour and 15 minutes with occasional stirring, after which the sample is filtered and the residue washed with hot distilled water. The sample is then ready for the sufite treatment as outlined by Matrone *et al.*

It was found that digestion and removal of starch in this manner greatly facilitated filtration of many samples, without adversely affecting the cellulose determination. The mylase-buffer solution is made as follows:

One hundred and sixty-four grams of anhydrous sodium acetate are dissolved in 125 ml. of glacial acetic acid and the mixture is diluted to 1 liter with distilled water to give a pH of 4.5. For each day's determinations, one gram of Mylase P is added to 25 ml. of buffer solution and 3 ml. of this is used for the digestion of each sample.

The lignin analyses were made by the method of Ellis, Matrone and Maynard (1946), also modified to suit our conditions as described in the following:

After the acid pepsin digestion, the sample is filtered and washed

with distilled water. One hundred ml. of 0.25 percent sodium carbonate are added and the sample is digested at 39° C. overnight. After filtration and washing, the sample is ready for the first acid treatment according to the method of Ellis *et al.* Pig feces treated under our conditions (dried at 50°–60° C.) retained sufficient proteolytic activity in alkaline pH so that the customary addition of trypsin to the reaction mixture was not necessary.

In using the unmodified method (Ellis, 1946) for lignin determination, negative digestibilities of the order of 25–40 percent were frequently obtained. Our modification permitted more reasonable results. It is probable that the "extra" lignin found in the feces by the original method consists largely of protein. The incomplete removal of nitrogenous matter from feces by acid pepsin digestion is illustrated by the following experiment.

Feces were obtained from a calf on synthetic milk (roughage-free) diet and were analyzed for lignin by the method of Ellis *et al.* The average of nine determinations was 5.40 percent. Three samples of the same feces were carried through the lignin determination up to the point of ashing. The residue of lignin plus ash was analyzed for total nitrogen by the Kjeldahl method and the results, expressed as protein, averaged 5.14 percent. This appeared to account for most of the "lignin" in the feces of this animal. Additional samples of the same feces were analyzed for lignin by our modification; the average of four determinations was 0.32 percent lignin. Further tests with pig feces showed that the modified method yielded approximately 34 percent less protein ($N \times 6.25$) in the lignin fraction than did the original method. Hydrolysates of lignin isolated from pig feces were chromatographed on filter paper. The two-dimensional chromatograms showed spots representing most of the common amino acids. The spots representing the leucine group were especially strong, and microbiological assay of the hydrolysate for isoleucine indicated 2.2 percent of this amino acid in the original lignin. De Man and De Heus (1950) have detected tryptophane and tyrosine in preparations of lignin from grass.

Results and Discussion

The averaged data obtained in this investigation are presented in table 2 and figures 1 to 3. The statistical computations were made by Student's "t" test for paired observations.

The substitution of cellulosic materials for starch under the conditions of trials A, B, and C did not markedly affect the digestibility of total

nitrogen in the rations, although there was a tendency toward decreased digestibility. This would seem reasonable, since the metabolic fecal nitrogen would tend to be greater from pigs consuming the more fibrous rations.

TABLE 2. AVERAGE DATA AND STATISTICAL ANALYSES

		Digestion Coefficients					Metabolizable Energy	
Trial	Description	Total Nitrogen	Gross Energy	Crude Fiber	Cellulose	Lignin	Cal./gm.	Percent of Digestible Energy
	Woodflock	79.1	77.0	32.1	41.1	−2.3	2.88	95.6
A	Starch	79.6	82.7	46.1	57.0	1.7	3.08	95.6
	Difference	−0.5	−5.7	−14.0	−15.9	−4.0	−0.20	0
	P (N=6)	0.39	0.01	0.09	0.03	0.18	0.01	—
	Ruffex	79.9	78.4	43.5	46.3	15.8	2.96	95.1
B	Starch	80.6	83.5	54.5	58.8	7.6	3.09	94.9
	Difference	−0.7	−5.1	−11.0	−12.5	8.2	−0.13	0.2
	P (N=6)	−0.13	0.001	0.004	0.01	0.23	0.001	—
	Wheat Straw Pulp	76.0	79.8	71.8	67.6	6.9	2.96	94.7
C	Starch	78.0	81.8	46.9	46.2	13.2	3.02	95.1
	Difference	−2.0	−2.0	24.9	21.4	−6.3	−.06	0.4
	P (N=6)	0.04	0.09	0.002	0.006	0.18	0.29	—
	Alfalfa Meal	71.7	72.8	42.5	49.7	—	2.69	93.2
D	Woodflock	79.5	75.5	26.6	37.2	—	2.81	93.9
	Difference	−7.9	−2.7	15.9	10.5	—	−0.12	−0.7
	P (N=5)	0.001	0.003	0.001	0.001	—	0.003	0.05
	Wheat Straw	73.3	71.0	27.4	40.0	−0.9	2.71	98.4
E	Woodflock	74.9	77.3	40.7	49.3	0.9	2.94	98.2
	Difference	−1.6	−6.3	−13.3	−9.3	−1.8	−0.23	0.2
	P (N=6)	0.11	0.001	0.06	0.14	0.29	0.001	—
	Oat Hulls	79.0	72.1	28.6	25.0	—	2.74	98.5
F	Woodflock	77.2	76.7	36.3	39.5	—	2.94	98.6
	Difference	1.8	−4.6	−7.7	−14.5	—	−0.20	0.1
	P (N=6)	0.03	0.001	0.11	0.07	—	0.001	—
	Alfalfa Meal + Sulfathalidine	71.6	70.5	29.3	36.6	0.4	2.71	98.0
G	Alfalfa Meal	74.9	74.4	40.9	49.4	4.0	2.84	98.2
	Difference	−3.3	−3.9	−11.6	−12.8	−3.6	−0.13	0.2
	P (N=5)	0.02	0.004	0.001	0.004	0.10	0.01	—

The digestible energy was significantly decreased by the substitution of woodflock and of Ruffex for starch. This was not the case when wheat straw pulp was used. These findings are justified by the calculation of digestibility of the added cellulose by the difference procedure; the percentages were 21, 30, and 92, respectively, when the source of cellulose was woodflock, Ruffex, or wheat straw pulp.

The digestibilities of total crude fiber and of total cellulose contained in the rations exhibit similar trends and are significantly affected by the changes in ration constituents with the exception to be seen in the

table. The differences in cellulose digestibility may be associated with lignification of the cellulose, as in Ruffex, or with difference in type of cellulose. The method of cellulose analysis used includes hemi-cellulose; the relative digestibility of cellulose and hemi-cellulose may differ (Mangold, 1934), although the matter is controversial (Ferguson, 1942;

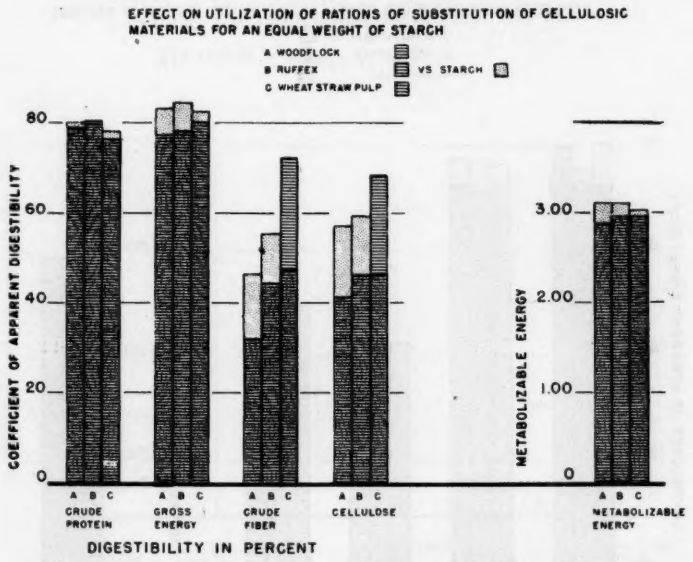

Figure 1. Showing changes in apparent digestibility (percent) and in metabolizable energy (Cal. per gram) of rations brought about by replacing 7.2 percent of starch by an equal amount of cellulosic materials.

McAnally, 1942). Such determinations have not yet been reported with respect to swine.

The size of the cellulose chain might also influence digestibility. It is probable that the alkali treatment in manufacture of wheat straw pulp affects the cellulose, in addition to removing lignin, in a manner to render it more readily available to microorganisms in the intestinal tract. The most likely explanation for the high digestibility of wheat straw pulp cellulose compared to that of woodflock is that in the latter case the cellulose has not been so affected by the sulfite pulping process as to render it completely available to the bacteria in the cecum and colon. Tests of the effect of alkali treatment of woodflock

on its digestibility should be undertaken, as well as tests of the sulfite treatment of wheat straw.

The digestibility of lignin contained in all rations was low, variable, and not obviously affected by the conditions of this experiment. The mean digestibility of lignin was 5.1 percent $\pm$ 1.14.

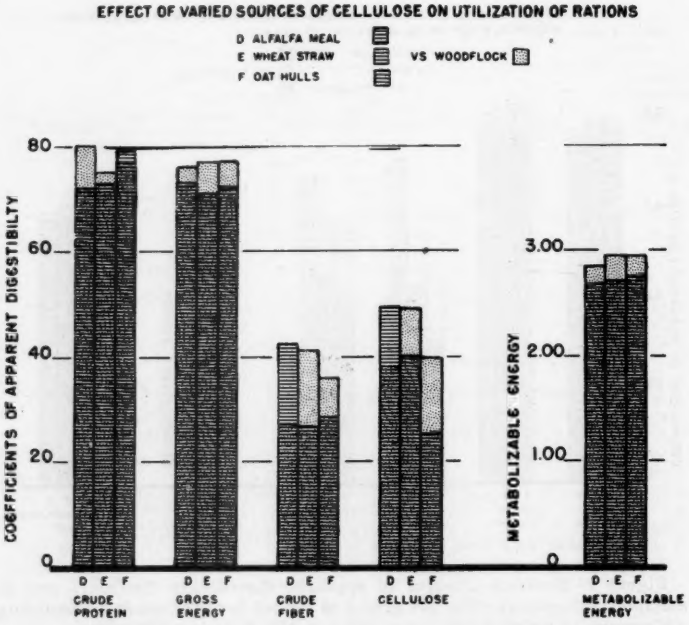

Figure 2. Showing changes in apparent digestibility (percent) and in metabolizable energy (Cal. per gram) of rations brought about by replacing cellulose of woodflock by an equivalent amount of cellulose in the form of other sources of cellulose.

The substitution of cellulosic materials for starch affected metabolizable energy, expressed in Calories per gram, in the same manner as it affected the digestibility of the gross energy. This may be seen from the calculation of the metabolizable energy, as a percentage of the digestible energy, which indicates that the absorbed products of cellulose and pentosan fermentation are useful as energy sources to the animal. The digestible and the metabolizable energy data represent apparent values rather than true ones, due not only to the metabolic

and endogenous losses of energy, but also to the production of volatile gases in cellulose fermentation. Correction for gaseous losses of energy would slightly increase the metabolizable energy differences between high starch and high cellulose rations.

The next three trials were designed to investigate the effect of sub-

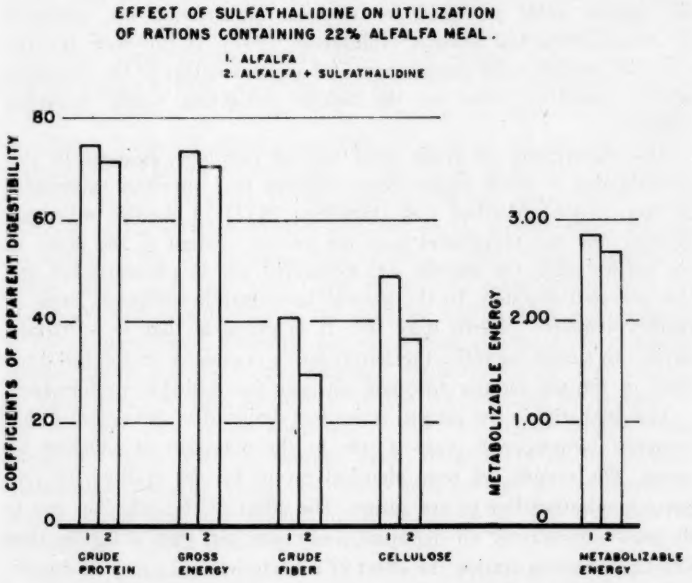

Figure 3. Showing changes in apparent digestibility (percent) and in metabolizable energy (Cal. per gram) of rations brought about by feeding 1.85 percent sulfathalidine.

stituting a variety of cellulosic materials for woodflock, all substitutions being made on an equal cellulose basis.

The decrease of total nitrogen and of energy digestibility observed as a result of substitution of alfalfa meal for woodflock is probably due in large part to the unavailability of nitrogen and other nutrients in the alfalfa meal cells. Although the digestibility of alfalfa meal fiber and cellulose was greater than of woodflock, the latter cellulose did not enclose important quantities of other nutrients.

The substitution of wheat straw for woodflock significantly lowered only the digestibility of gross energy, although the other digestion

coefficients showed the same trends. The slight effect on total nitrogen digestibility is traceable to the low amount of protein in wheat straw.

The substitution of oat hulls for woodflock significantly decreased only the digestibility of energy.

The data showing a similar degree of utilization of the digestible energy in all comparisons support findings of other investigators that the organic acids produced in cellulose fermentation are utilizable by simple-stomached animals (Phillipson, 1947). If this were not the case, the metabolizable energy expressed as a percentage of the digestible energy would be lower for the rations containing highly digestible cellulose.

The digestibility of crude fiber and of cellulose obtained in this investigation is much higher than obtained in a previous experiment at this station (Mitchell and Hamilton, 1932). A notable difference between the two procedures is in the protein content of the diets. In the earlier trials the protein was extremely low in concentration and was not well digested. In the present investigation adequate levels of readily available protein were fed. It is probable that in a manner similar to rumen bacteria, the fermentative organisms in the intestinal tract of the pig require adequate nitrogen for optimum performance.

The final trial in the present series was designed to demonstrate that bacterial fermentation plays a role in the digestion of cellulose by swine. The rations fed were identical except for the addition of 1.85 percent sulfathalidine to one ration. The effect of this addition was to decrease significantly all digestion coefficients but that of lignin, thus effectively demonstrating the effect of a bacteriostatic agent on digestibility of cellulose as well as the effect of an indigestible ligno-cellulose cell wall on the digestibility of other nutrients.

A further significant finding of this investigation is that the crude fiber and the cellulose utilization data are similar but in general indicate a greater utilization of cellulose. The cellulose plus lignin values were in considerable excess of the crude fiber values, the latter averaging only 72 percent $\pm$ 3.0 [3] of the former, while the crude fiber amounted to 84 percent $\pm$ 3.0[3] of the cellulose alone. For critical work the advantage of making the cellulose and lignin determinations in place of the crude fiber estimation is obvious. These data are in agreement with those of Nordfeldt *et al.* (1949).

[3] Standard error.

Summary

These studies were conducted to determine the effect of source of "crude fiber" on its digestibility and degree of utilization by swine and to investigate the advantages of substituting analyses for cellulose and lignin in place of the usual crude fiber estimation. Ruffex, wheat straw pulp, alfalfa meal, and oat hulls, were added to a good basal ration in amounts to give equivalent cellulose values. Determinations of digestibility and metabolizable energy were made, using Duroc Jersey and Poland China barrows weighing initially 125 pounds. Digestibility of cellulose in rations containing about 50 percent of their cellulose from woodflock, Ruffex, or wheat straw pulp averaged 41, 46, and 68 percent, respectively. The added cellulose in the same series was apparently digested to the extent of 21, 30, and 92 percent, respectively. Comparisons of varied fiber sources with woodflock showed the following decreasing order of cellulose digestibility: alfalfa meal, woodflock, wheat straw, and oat hulls. The differences cited above may be associated with lignification of the cellulose and/or chemical variation in the cellulose itself. Cellulose and crude fiber digestibilities exhibited similar trends, but the former exceeded the latter. Crude fiber content of the rations averaged 84 percent of the cellulose and 72 percent of the cellulose plus lignin contents. A modified lignin analysis was developed which gives an average lignin digestibility of 5.1 percent. Metabolizable energy values, expressed as a percent of the digestible energy, show that the organic acids produced in the digestion of cellulose are highly utilizable by the animal.

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THE EFFECT OF THREE FORMULATIONS OF BENZENE HEXACHLORIDE ON THE FLAVOR OF PORK[1]

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University of Illinois[2]

THE use of benzene hexachloride to control hog mange and lice has proved very effective and has become very popular with swine growers. Its effect on the meat of swine and poultry, however, has been a matter of much discussion. Hixson and Muma (1947) found that poultry meat may be rendered inedible after birds or poultry houses are treated with the chemical. The studies of Milby and Lawatsch (1948) and Harwood (1948) reveal that the use of oil emulsions of 1.25 to 1.50 percent gamma isomer benzene hexachloride had no effect on the flavor of poultry meat and eggs. Loeffel (1948) reported that meat from chickens kept overnight in a house sprayed 5 months previously with benzene hexachloride was inedible when the chickens were slaughtered the next day. He also reported that there was no objectionable flavor in pork from hogs slaughtered two weeks after spraying with benzene hexachloride. Lehrer *et al.* (1949) found no off-flavor in the meat of swine treated with a water suspension spray, made of a mixture of 20 pounds of 10 percent gamma isomer benzene hexachloride in 100 gallons of water, two or more weeks before slaughter. Grummer *et al.* (1950) reported that pork from hogs treated with liberal applications of benzene hexachloride dust and spray was not contaminated when each hog received approximately 2 ounces benzene hexachloride dust containing 1 percent gamma isomer or approximately 3 quarts of a solution of benzene hexachloride composed of 40 pounds of 6 percent gamma isomer benzene hexachloride per 100 gallons of water, more than 10 days before slaughter. However, treating animals immediately prior to slaughter caused an off-flavor in the lean and fat.

Experimental Methods

Twenty hogs of good to choice grade weighing about 225 pounds at slaughter were used for this test. They were of the Hampshire, Poland China, and Chester White breeds. They were divided into four groups. Group 1, the control group, contained eight hogs and was not sprayed.

[1] This experiment was supported by a grant of funds and three formulations of benzene hexachloride to the University of Illinois by Commercial Solvents Corporation, Terre Haute, Indiana.

[2] Urbana.

The other groups contained four hogs each and were treated as follows:

Group 2. Sprayed with a 10 percent gamma isomer emulsifiable solution of technical benzene hexachloride in methyl ethyl ketone solution.

Group 3. Sprayed with a 15 percent gamma isomer emulsifiable solution of Lindane (99 percent gamma isomer of benzene hexachloride) in methyl ethyl ketone solution.

Group 4. Sprayed with 12 percent gamma isomer technical benzene hexachloride wettable powder.

All products were diluted with water to 0.125 percent gamma isomer concentration and were applied to the hogs with a hand operated pressure sprayer until the hair and skin were apparently saturated.

Two hogs from each sprayed group were slaughtered one day after being sprayed, and one hog from each sprayed group was slaughtered at seven and at fourteen days after being sprayed. One or more control hogs were slaughtered each time.

A center-cut loin roast containing an inch of fat was cut from the right side of each carcass. The roasts were cooked and served without seasoning in the home economics laboratory according to specifications of the Cooperative Meat Investigators (1927). A palatability committee consisting of six to eight members judged the fat, lean, and drippings of the roasts. The results were analyzed statistically by the method of chi-square by comparing the roasts of the sprayed groups with roasts from the control group.

Results and Discussion

Table 1 gives a summary of the results. Each member of the palatability committee scored the fat, lean, and drippings of each roast for aroma and flavor as desirable, slightly undesirable and distinctly undesirable; therefore, each roast was scored six times. The total score for any roast is six times the number of judges. The scores are converted to a standard basis to eliminate the difference in numbers of judges by converting them to a percentage basis. The results from roasts taken from the eight control hogs are averaged and used as the expected value and are compared with the results obtained from roasts from the differently sprayed groups. Group 3 slaughtered one day after treatment were as desirable or more desirable than the control roasts. Individuals variations in scoring are indicated in cases where the roasts from sprayed hogs are significantly more desirable than the control roasts. The roasts from one of the hogs of group 2 slaughtered

TABLE 1. SUMMARY OF PALATABILITY TESTS

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Spray Treatment	Days sprayed before slaughter	Number of judges	Results[1]					
			Desirable		Slightly Undesirable		Distinctly Undesirable	
			Score	Percent	Score	Percent	Score	Percent
Controls		7	22	52.4	14	33.3	6	14.3
No treatment		6	29	80.6	7	19.4	0	0
		6	21	58.3	14	38.9	1	2.8
		6	21	58.3	12	33.3	3	8.3
		7	41	97.6	1	2.4	0	0
		8	40	83.3	6	12.5	2	4.2
		7	41	97.6	1	2.4	0	0
		8	35	72.9	13	27.1	0	0
Average Control				75.1		21.2		3.7
Group 2. Methyl	1	7	22	52.4	12	28.6	8	19.0
ethyl ketone solution	1	6	24	66.7	10	27.8	2	5.6
of technical BHC	Av. 1 day			59.6		28.2		12.3
	7	6	29	80.6	6	16.7	1	2.8
	14	7	37	88.1	3	7.1	2	4.8
Group 3. Methyl	1	7	36	85.7	6	14.3	0	0
ethyl ketone solution	1	6	28	77.8	6	16.7	2	5.6
of Lindane	Av. 1 day			81.8		15.5		2.8
	7	6	33	91.7	3	8.3	0	0
	14	7	42	100.0	0	0	0	0
Group 4.	1	7	16	38.1	11	26.2	15	35.7
Technical BHC	1	6	14	38.9	15	41.7	7	19.4
wettable powder	Av. 1 day			38.5		34.0		27.5
	7	6	34	94.4	2	5.6	0	0
	14	7	36	85.7	4	9.5	2	4.8

[1] As each roast was scored six times by each judge the total score for each roast is six times the number of judges. The scores are converted to a standard percentage basis to eliminate the variation due to different numbers of judges.

one day after treatment was significantly less desirable than the control roasts. However, this difference was no greater than some differences in favor of roasts from treated hogs as compared with control roasts; therefore, it is reasonable to assume that this lack of desirability could be due to individual variation in scoring.

Roasts from both hogs of group 4 slaughtered one day after treatment were much less desirable than the control roasts. If we assume that 100 percent desirability, as indicated by the roast from the hog of group 3 slaughtered 14 days after treatment, is indicative of the largest normal variation, the differences are still highly significant at the .01 level. This indicates that roasts from hogs of group 4 slaughtered one day after treatment contain off-flavors and off-odors due to the technical benzene hexachloride in wettable powder form.

Summary and Conclusions

Four groups of 20 good to choice grade hogs weighing about 225 pounds of Hampshire, Poland China, and Chester White breeding were treated as follows: Group 1 was not sprayed. Group 2 was sprayed with a 10 percent gamma isomer emulsifiable solution of technical benzene hexachloride in methyl ethyl ketone. Group 3 was sprayed with a 15 percent gamma isomer emulsifiable solution of Lindane in methyl ethyl ketone. Group 4 was sprayed with a 12 percent gamma isomer technical benzene hexachloride wettable powder. All materials were diluted to 0.125 percent gamma isomer concentration. The hogs were slaughtered at intervals ranging from one to fourteen days after being treated. Loin roasts were cooked and palatability tests were conducted on the roasts.

The following conclusions were reached:

1. Pork from hogs sprayed with a 0.125 percent Lindane solution one day before slaughter was affected none by the treatment.
2. Pork from hogs sprayed with a 0.125 percent gamma isomer BHC technical as wettable powder slaughtered seven days after treatment was not affected. One day after spraying was not sufficient to obtain a score equal to that of the control.
3. Dissolving technical BHC in methyl ethyl ketone eliminated some of the undesirable effects. Pork from hogs sprayed with this preparation and slaughtered one day later was better than that from hogs sprayed with benzene hexachloride technical as wettable powder. However, it was not as acceptable at one day after spraying as pork from hogs sprayed with Lindane.

4. Pork from hogs sprayed one week or more before slaughter with any of the benzene hexachloride preparations tested scored as well or better than the controls in palatability.

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THE USE OF FECAL NITROGEN AS A MEASURE OF DRY MATTER INTAKE AND OF DIGESTIBILITY OF ORGANIC MATTER IN FORAGE

T. Homb and K. Breirem

The Agricultural College of Norway[1]

RECENTLY several attempts have been made to determine the dry matter intake of grazing animals on the basis of the nitrogen excretion in the feces. Further, the fecal nitrogen excretion has also been proposed as a measure of the digestibility of organic matter in herbage (Gallup and Briggs, 1948; Lancaster, 1949). In both cases the calculation methods are based upon the assumption that the fecal nitrogen excretion forms a constant percentage of the dry matter intake. The ratio of fecal nitrogen excretion to dry matter intake seems, however, to increase somewhat with increasing protein content in the ration (Gallup and Briggs, 1948; Blaxter and Mitchell, 1948; Lancaster, 1949b). If this variation in the ratio is significant, there should be good reason for using a corrected value of the ratio of fecal nitrogen to dry matter intake in calculating the feed intake of grazing animals (Forbes, 1949).

Experimental

During the years 1944–49 digestion experiments with 52 different qualities of artificially dried timothy-clover mixture (up to 45 percent clover) have been carried out at the Agricultural College of Norway. The electrical drying has been done immediately after harvesting, and the drying temperature varied from 70° to 80° C. Of the samples 22 were of first cut (May–June), 25 second (July–August), and 5 third cut (about Oct. 1). The experiments have been carried out on adult sheep, and the feces have been collected quantitatively during a 12-day period. Six of the 52 samples have been used in a single experiment with one sheep, while the digestibility of the remaining 46 samples has been determined in duplicate tests.

Results and Discussion

As to the 22 samples of first cut the following significant rectilinear regression of the fecal nitrogen excretion in percentage of the dry

[1] Division of Animal Nutrition.

matter intake (y) on the crude protein content of the herbage (dry basis) (x) has been found:

$Y=0.452+0.00935\,x$ $r=0.81$

A similar analysis based on the 30 samples of the second and third cuts gives the following equation:

$Y=0.264+0.03214\,x$ $r=0.80$

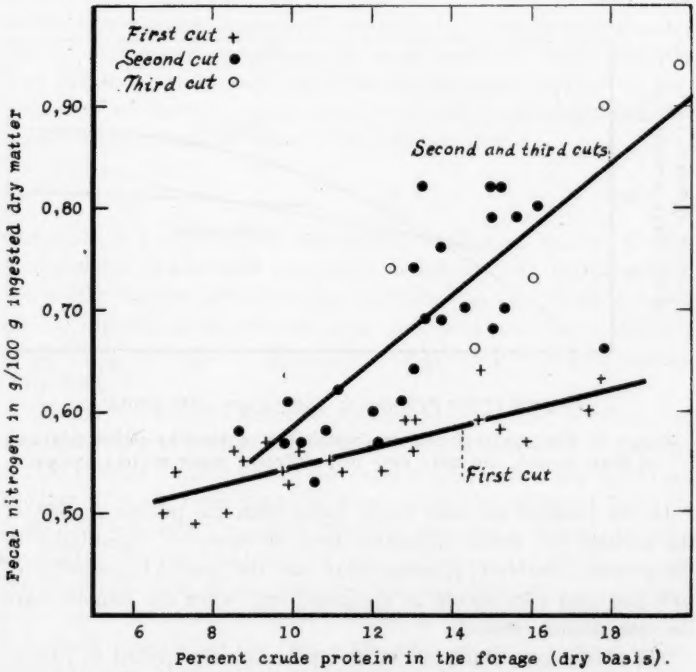

Figure 1. Fecal nitrogen per 100 gm. ingested dry matter at different protein content in the forage.

Figure 1 shows a distinct difference between the regression line based upon first cut and the line based on second and third cuts. The average fecal nitrogen output in grams per 100 grams of dry matter intake for the different cuts was: first cut, 0.56; second cut, 0.68; third cut, 0.79.

These results indicate the importance of (1) distinguishing between the first cut and the aftermath when determining the ratio of fecal-

nitrogen excretion to dry matter intake and (2) of taking the protein content of the herbage into consideration.

The difference between first and later cuts in this respect depends upon a lower apparent digestibility of the protein in aftermath. This appears clearly in figure 2 where the apparent crude protein digestibility is given as a function of the crude protein content of the feed on the dry basis. The curves have been plotted by means of the method of least squares.

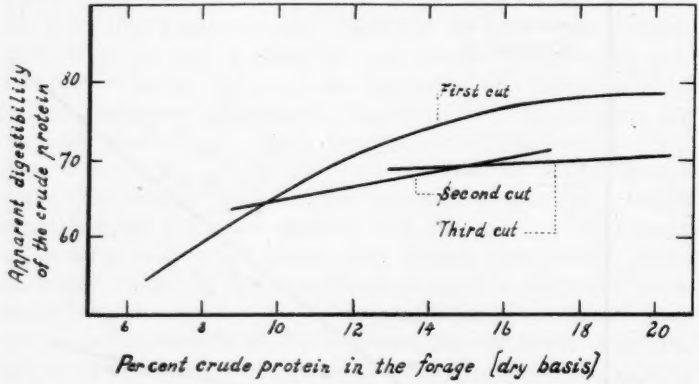

Figure 2. The apparent protein digestibility of timothy-clover mixtures of first, second, and third cuts with different crude protein content.

In our material no other single factor than the protein content of the herbage has greater influence upon the apparent digestibility of the protein. Therefore, it seems clear that the protein digestibility of first and later cuts should be compared only when the samples have the same protein content.

The results here obtained can hardly be directly applied to pasture grass. The samples have been obtained at different stages of growth, from a somewhat late grazing stage up to the flowering of the timothy plant. However, it seems reasonable to have some doubt as to the constancy of the ratio of fecal nitrogen output to dry matter intake in pasture herbage throughout the whole season, even when the protein content is the same. If this doubt were justified, fecal nitrogen excretion would not be a good measure for the feed intake of grazing animals, especially if we are not able to make correction for different periods of the pasture season. The data presented by *Forbes* (1949) indicate

that the ratio of fecal nitrogen excretion to dry matter intake of grazing sheep is subject to considerable variation. He could find no significant relation to the protein content of the forage. We shall not try to relate this to seasonal differences, though they may have been a contributory reason for the heterogeneous results.

Lancaster (1949) has shown how to deduce the equation for calculating the digestibility of organic matter in pasture herbage on the basis of the nitrogen content in the feces. He prefers to base his calculation on the fecal-nitrogen excretion in percentage of organic matter rather than of dry matter, considering the dusty nature of forage crops in New Zealand. The average figure for fecal-nitrogen excretion in percentage of ash-free dry matter intake was 0.83. Supposing this figure to be constant, Lancaster deduced the following formula:

$$D_n = \frac{100 \cdot n - 100 \cdot C}{n}$$

where D_n is the calculated digestibility of organic matter, C=the fecal-nitrogen excretion in percentage of ash-free dry matter intake, and n=the nitrogen percent in ash-free feces. This formula gives values for the digestion coefficients in close agreement with the coefficients obtained by the orthodox method, but for samples with lower digestibility than 70.

The above-mentioned Lancaster formula has been used in calculating the digestibility of organic matter in each of our samples. For the first cut the average C-value will be 0.60, and for the second and third cuts together 0.73. On replacing C by these values the estimated digestion coefficients will show a standard deviation from the coefficients actually obtained in the experiments of 2.54 units for first cut and 4.54 for second and third cuts. Also in our material there is a clear tendency towards a greater deviation when the digestibility is low.

A similar calculation has been made when giving C varying values according to the crude protein content of the dry matter in each sample. The C-value has been derived from the following equations:

First cut	$C=0.458+0.0123\ x$
Second and third cuts	$C=0.254+0.0366\ x$

where x equals percentage of crude protein in the herbage on the dry basis.

On use of this method the estimated digestion coefficients of organic matter in the first cut of herbage show a standard deviation of 1.32 units from the observed figures and of 2.61 units for second and third

cuts. Thus, the deviation is considerably smaller than was the case when correction was not made for the protein content of the forage.

In both cases the average calculated digestibility for all samples showed close agreement with that obtained in the experiments.

Summary

In estimating the digestibility of the organic matter of a single sample of herbage on the basis of the fecal nitrogen it seems to be of great importance to consider the content of protein in the feed. Another main point is to differentiate between first and later cuts. Taking these points into consideration, Lancaster's formula may give estimated digestion coefficients that agree fairly well with those obtained by the orthodox method. This seems especially to be true for the first harvesting in early summer.

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BALANCING THE NUTRITIONAL DEFICIENCIES OF ROUGHAGES FOR BEEF STEERS[1]

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ONE method of improved efficiency in beef production is through improved efficiency of converting high cellulose feeds such as corn cobs, soybean straw, oat straw, corn stalks, cottonseed hulls, corn silage and grass silage into a highly efficient growing ration. At present a large proportion of these roughages are being wasted through improper use. Each year, for instance, over twenty million tons of corn cobs are produced annually, but by far the larger share is burned or thrown out to rot (Lathrop and Shollenberger, 1947).

Today a new concept is being developed in the feeding of cattle which is based on the evidence that in order to properly feed ruminants on roughages, such as corn cobs, straw or silage, a supplement must be designed to contain adequate nutrients, properly balanced, to nourish the billions of bacteria in the rumen or paunch of cattle, so the indigestible roughages can be broken down into a usable form (Burroughs *et al.*, 1949a, 1949b, 1949c, 1950a, 1950b, 1950c, 1950d, 1950e; Swift *et al.*, 1951).

The growth response of steer calves and yearlings is affected greatly by the balance of energy, protein, vitamins and minerals available to the animal in its daily feed. It appears likely that poorer growth responses which have been reported in former years from the feeding of common farm roughages have been due to a deficiency of, or improper balance of, nutrients required by rumen microorganisms. Therefore, these experiments were designed to re-evaluate the common farm roughages, when fortified with various nutrients, apparently required by these micro forms of life present in the rumen.

Experimental

A series of experiments were set up to study the growth response of steer calves and yearlings to the various roughage supplementation programs outlined.

In all feeding trials reported, there was available at all times a simple mixture composed of two parts steamed bone meal to one part of

[1] Contribution from the Department of Animal Husbandry, Journal Paper No. 605, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

salt. In addition, salt was available at all times. In the supplement fed lots, steamed bone meal and salt containing one ounce of cobalt sulfate per 100 pounds salt was incorporated into the supplement at levels recommended by the National Research Council.

Corn cobs were ground through a hammer mill using a half inch screen. In experiment I, all the ingredients fed in corn-cob lots were mixed with the cobs with the exception of the cane molasses, which was diluted with an equal volume of water and poured over the cob mixture at feeding time. In all experiments subsequent to Experiment I, roughages being tested were poured in the troughs twice daily and the supplemental mixture containing molasses, protein supplement, minerals and vitamins were poured over the roughage and mixed in with a silage fork.

Description of feed ingredients

Vitamin A and D concentrate—a concentrated cod liver oil of a potency of 2250 I.U. of A and 300 I.U. of D per gram.

Cane molasses—ordinary blackstrap molasses.

Alfalfa meal—dehydrated alfalfa meal containing 17 percent crude protein.

Soybean meal—expeller, 41 percent crude protein.

Molasses Feed—a 45 percent molasses feed in which the molasses has been dried on oat hulls and screenings.

Urea—262 percent crude protein equivalent.

Distiller's dried solubles—dried distillers solubles product fortified with Vitamin A and D. Crude protein content—24 percent. (*15000 I.U.* A and *5000 I.U.* D per pound).

Dried Brewer's yeast—45 percent crude protein.

Corn silage—made from 70 bushel corn, yielding from 14 to 15 tons of green material per acre.

Chopped oat straw—ordinary oat straw was run through a hay chopper as needed.

Active dry yeast—a live cell yeast of which the activity was equivalent to twenty billion cells per gram.

Vitamin B_{12} supplement—a B_{12} supplement with a potency of 12.5 milligrams per pound (L.L.D. cup assay). No antibiotic potency.

Chopped soybean straw—soybean straw as picked up behind the combine was run through an ordinary hay chopper as needed.

Grass silage—first cutting alfalfa (approximately 90 percent alfalfa

and 10 percent Ladino clover) was cut with a field chopper and blown into the silo freshly cut from the field without any wilting. The fresh green material yielded 13.5 tons per acre and comparable alfalfa yielded three tons of hay per acre. Two hundred pounds of ground shelled corn was added per ton of silage as a preservative.

Purdue Cattle Supplement A

TABLE 1. COMPOSITION OF PURDUE CATTLE SUPPLEMENT A

	1000 Lb. Mix	Per steer, daily
Soybean Oil meal	643.1	2.25
Molasses (45% Molasses Feed)[1]	285.8	1.00
Bonemeal	51.4	.18
Salt	17.2	.06
Vitamin A and D concentrate[2]	2.5	.01
Total	1000.0	3.50

[1] 45 percent molasses dried on oat hulls and screenings.
[2] 2250 A—300 D per gram fortified cod liver oil.

Experiment I (1948–1949) November 5, 1948, to April 22, 1949—168 days

Ninety-six Texas Hereford steer calves were divided as equally as possible according to type and initial weight into six dry lots with 16 calves per lot, and the following rations were fed daily per steer:

Lot I	Per steer, daily lb.
Mixed clover-timothy hay	20.00
Minerals	free choice
Lot II	
Mixed clover-timothy hay	14.00
Ground shelled corn	4.00
Minerals	free choice
Lot III	
Mixed clover-timothy hay	14.00
Ground ear corn	4.00
Minerals	free choice
Lot IV	
Ground corn cobs	16.00
Soybean oil meal	2.50
Cane molasses	1.00
Bone meal	.18
Salt	.06
Vitamin A and D concentrate	17 grams
Minerals	free choice

Lot V	
Ground corn cobs	15.00
Alfalfa meal	1.00
Soybean meal	2.50
Cane molasses	1.00
Bonemeal	.18
Salt	.06
Minerals	free choice
Lot VI	
Ground corn cobs	14.00
Alfalfa meal	2.00
Soybean meal	2.50
Molasses	1.00
Bonemeal	.18
Salt	.06
Minerals	free choice

Experiment II (1949–50) November 4, 1949, to May 17, 1950, 198 days

One hundred twelve Colorado Hereford short yearling steers were divided into seven lots of 16 steers each and received the following rations daily:[2]

Lot I	Per steer, daily lb.
Mixed clover-timothy hay	free choice
Minerals	free choice
Lot II	
Ground corn cobs	free choice
Purdue Cattle Supplement A	3.50
Minerals	free choice
Lot III—One third of protein met with urea	
Ground corn cobs	free choice
Soybean meal	1.40
Ground corn	.84
Molasses Feed	1.00
Urea	.12
Bonemeal	.18
Salt	.06
Vitamin A and D concentrate (4 grams)	.01
Total supplement	3.61
Minerals	free choice

[2] All supplements were adjusted to the same energy and protein equivalent.

Lot IV—Distillers dried solubles	
Ground corn cobs	free choice
Distillers dried solubles	2.00[3]
Urea	.18
Molasses feed	1.00
Ground corn	.34
Bonemeal	.18
Salt	.06
Total supplement	3.76
Minerals	free choice
Lot V—Dried Brewer's Yeast	
Ground corn cobs	free choice
Soybean meal	2.00
Brewer's yeast	.18
Molasses feed	1.00
Ground corn	.22
Bonemeal	.18
Salt	.06
Vitamin A and D concentrate (4 grams)	.01
Total supplement	3.65
Minerals	free choice
Lot VI—Alfalfa meal added	
Ground corn cobs	free choice
Soybean meal	1.48
Corn	.65
Alfalfa meal	2.00[4]
Molasses feed	1.00
Bonemeal	.18
Salt	.06
Total supplement	5.37
Minerals	free choice

Lot VII. Fattening ration started same as Lot VI. Gradually corn was added, replacing some of the corn cobs until the steers were on full feed. They consumed an overall average of 11.03 pounds of corn per steer, daily.

Experiment III (1949–1950) December 21, 1949 to May 3, 1950—133 days

One hundred and one Colorado and Montana Shorthorn steer calves were divided into three dry lots of 33, 34 and 34 calves per lot. All lots received 3.5 pounds of Purdue Cattle Supplement A per steer per day plus one of the three roughages listed. The roughage was placed in

[3] No Vitamin A and D concentrate was added because distillers dried solubles supply these vitamins in excess of the recommendation of the National Research Council.

[4] Alfalfa meal contributed sufficient vitamin A, eliminating the cod liver oil.

the troughs twice daily, the supplement was poured over the roughage and the supplement was mixed with a fork. In addition, all animals had free access to the simple mineral mixture listed previously.

	Per steer, daily lb.
Lot I	
Chopped oat straw	free choice
Purdue Supplement A	3.5
Minerals	free choice
Lot II	
Ground corn cobs	free choice
Purdue Supplement A	3.5
Minerals	free choice
Lot III	
Corn silage	free choice
Purdue Supplement A	3.5
Minerals	free choice

Experiment IV (1950–1951) November 8, 1950, to May 2, 1951—175 days

Ninety-eight Wyoming Hereford Yearling steers were separated into seven dry lots of 14 steers each. Each lot of steers received ground corn cobs free choice, twice daily. The following supplemental mixtures were poured over the cobs of the respective lots and mixed in with a fork at feeding time.[5]

	Per steer, daily lb.
Lot I	
Ground corn cobs	free choice
Purdue Supplement A	3.5
Minerals	free chioce
Lot II—one-third of protein from urea	
Ground corn cobs	free choice
Soybean meal	1.06
Molasses feed	2.85
Urea	.138
Bonemeal	.18
Salt	.06
Vitamin A and D concentrate (4 grams)	.01
Total supplement	4.30
Minerals	free chioce

[5] All supplements were adjusted to the same energy and protein equivalent.

	Per steer, daily lb.
Lot III—one-half of protein from urea	
Ground corn cobs	free choice
Soybean meal	.46
Molasses feed	3.75
Urea	.209
Bonemeal	.18
Salt	.06
Vitamin A and D concentrate (4 grams)	.01
Total supplement	4.67
Minerals	free chioce
Lot IV—Two-thirds of protein from urea	
Ground corn cobs	free choice
Molasses feed	4.46
Urea	.263
Bonemeal	.18
Salt	.06
Vitamin A and D concentrate (4 grams)	.01
Total supplement	4.97
Minerals	free chioce
Lot V—one-fourth of protein from fish meal	
Ground corn cobs	free choice
Soybean meal	1.55
Fish meal	.43
Molasses feed	1.48
Bonemeal	.18
Salt	.06
Vitamin A and D concentrate (4 grams)	.01
Total supplement	3.71
Minerals	free chioce
Lot VI—active dry yeast	
Ground corn cobs	free choice
Soybean meal	2.25
Molasses feed	1.00
Active dry yeast	.018
Bonemeal	.18
Salt	.06
Vitamin A and D concentrate (4 grams)	.01
Total supplement	3.52
Minerals	free chioce

Lot VII—Vitamin B_{12}	
Ground corn cobs	free choice
Soybean meal	2.25
Molasses feed	1.00
Vitamin B_{12} supplement	.018
Bonemeal	.18
Salt	.06
Vitamin A and D concentrate (4 grams)	.01
Total supplement	3.52
Minerals	free chioce

Experiment V (1950–1951) December 8, 1950 to May 4, 1951—147 days

Eighty Nebraska and Montana Shorthorn calves were divided into four dry lots of 20 calves per lot and were fed the following rations.

Lot I	Per steer, daily lb.
Chopped soybean straw	free choice
Purdue Supplement A	3.5
Minerals	free choice
Lot II	
Ground corn cobs	free choice
Purdue Supplement A	3.5
Minerals	free choice
Lot III	
Grass silage	free choice
Purdue Supplement A	3.5
Minerals	free choice
Lot IV	
Corn silage	free choice
Purdue Supplement A	3.5
Minerals	free choice

Results

Experiment I

The results of this trial are summarized in table 2. Forty-eight head of steer calves (Lots IV, V, and VI) were wintered successfully for 168 days using corn cobs as the only or principal source of roughage. Steers fed corn cobs supplemented with soybean meal, molasses, minerals and cod liver oil gained an average of one pound per steer daily (Lot IV). This was .29 pound more per day than steers wintered with free access to clover-timothy hay.

Supplementing the corn cob ration with one (Lot V) or two (Lot VI) pounds of alfalfa meal increased the rate of growth in corn cob steers. Apparently alfalfa meal contains some nutritional factor or factors which aid in balancing a corn cob ration and stimulate growth in cattle beyond the extra energy that would be furnished by alfalfa meal. Burroughs *et al.* (1950) reported that a water extract of dehydrated

TABLE 2. WINTERING STEERS ON CORN COBS
November 5, 1948–April 22, 1949—168 days

Lot No.	Ration	Daily Feed, lb.	Feed per lb. gain, lb.	Initial Weight, lb.	Final Weight lb.	Gain per Steer, lb.	Daily Gain, lb.
I	Clover-timothy hay	20.00	27.92	552	673	121	0.72
	Minerals, free choice						
II	Gr. shelled corn	4.00	3.49	550	742	192	1.14
	Clover-timothy hay	14.00	12.36				
	Minerals, free choice						
III	Corn and cob meal	4.00	3.83	555	729	174	1.04
	Clover-timothy hay	14.00	13.60				
	Minerals, free choice						
IV	Corn cobs	16.00	15.74	551	721	170	1.01
	Soybean meal	2.50	2.54				
	Molasses	1.00	.93				
	Minerals, free choice						
V	Corn cobs	15.00	13.15	551	742	191	1.14
	Alfalfa meal	1.00	.82				
	Soybean meal	2.50	2.26				
	Molasses	1.00	.83				
	Minerals, free choice						
VI	Corn cobs	14.00	10.92	548	764	216	1.28
	Alfalfa meal	2.00	1.46				
	Soybean meal	2.50	2.00				
	Molasses	1.00	.73				
	Minerals, free choice						

alfalfa meal or the ash of the alfalfa meal fed at the rate equivalent to four pounds of meal daily per steer improved the digestion of corn cobs materially. Swift *et al.* (1951) reported similar results from the feeding of alfalfa meal ash with corn cobs to sheep.

There was no significant difference in the growth rate of steers receiving 4.0 pounds of ground shelled corn (Lot II) as compared to the group fed 4.0 pounds of corn and cob meal daily (Lot III). Apparently steers react differently from lambs because Bell (1949) concluded corn and cob meal was not as efficient as corn in the fattening ration of lambs.

Experiment II

The data as summarized in table 3, show rather conclusively that yearling steers will grow at a rate ranging from 1.19 to 1.33 pounds daily when corn cobs are used as the only roughage if the cobs are fortified with a protein supplement, molasses, minerals, and a vitamin A and D concentrate.

TABLE 3. WINTERING STEERS ON CORN COBS
November 4, 1949–May 17, 1950—198 days

Lot No.	Ration	Daily Feed, lb.	Feed per lb. gain, lb.	Initial Weight, lb.	Final Weight lb.	Gain per Steer, lb.	Daily Gain, lb.
I	Clover-timothy hay	20.09	22.8	595	767	172	.88
II	Corn cobs	14.70	11.5	592	843	251	1.28
	Supplement (Soybean Oil Meal)	3.63	2.8				
III	Corn cobs	14.64	11.5	590	840	250	1.27
	Supplement (Urea)	3.74	2.9				
IV	Corn cobs	14.5	12.2	591	824	233	1.19
	Supplement (Distillers Solubles Urea)	3.87	3.2				
V	Corn cobs	14.67	11.4	592	844	252	1.28
	Supplement (Brewers' Yeast)	3.77	2.9				
VI	Corn cobs	14.65	10.3	590	869	279	1.42
	Supplement (Alfalfa Meal)	5.50	3.9				
VII	*Fattening Ration*						
	Corn	11.03	4.8	592	1038	446	2.30
	Corn cobs	8.65	3.8				
	Supplement (Alfalfa Meal)	4.84	2.1				

Urea successfully replaced 1/3 of the protein of soybean meal when ground corn was added to replace the energy of the soybean meal replaced by urea. As in the previous trial, supplementing the ration with alfalfa meal resulted in increased daily gain of steers (Lot VI). The daily gain of 2.30 pounds per steer in Lot VII indicates steers may be fattened out on a ration whose only source of roughage is ground corn cobs.

Experiment III

The results of this phase are summarized in table 4. When fed 3.5 pounds of Purdue Cattle Supplement A (for composition, see table 1)

TABLE 4. OAT STRAW, CORN COBS AND CORN SILAGE FOR WINTERING STEER CALVES
December 21, 1949–May 3, 1950—133 days

Lot No.	Steers Per Lot	Av. Initial Wt. lb.	Av. Final Wt. lb.	Total Gain Per Steer, lb.	Av. Daily Gain, lb.	Av. Daily Feed		Feed per lb. gain	
						lb. Roughage	lb. Supp. A	lb. Roughage	lb. Supp. A
I Oat Straw Supp. A	33	485	588	103	0.78	12.4	3.5	16.0	4.5
II Corn cobs Supp. A	34	479	687	208	1.56	13.0	3.5	8.3	2.2
III Corn silage Supp. A	34	478	751	273	2.06	32.2	3.5	15.6	1.7

per day steers grew at the rate of 0.78 pounds per day when oat straw was the only roughage, 1.56 pounds per day when corn cobs was the only roughage, and 2.06 pounds per day when corn silage was the only roughage. In the corn silage lot the steers ate an average of 34 pounds of corn silage per steer daily and at no time gave evidence of looseness of bowel.

TABLE 5. WINTERING STEERS ON CORN COBS
November 8, 1950–May 2, 1951—175 days

Lot. No.		Daily Feed, lb.	Feed per lb. gain, lb.	Initial Weight, lb.	Final Weight, lb.	Gain per Steer, lb.	Daily Gain, lb.
I	Corn cobs	15.13	11.8	664	888	224	1.28
	Supplement (Purdue Cattle Supp. A)	3.49	2.7				
II	Corn cobs	14.49	11.6	670	889	219	1.25
	Supplement (1/3 protein from Urea)	4.28	3.4				
III	Corn cobs	14.63	12.8	668	868	200	1.14
	Supplement (1/2 protein from Urea)	4.66	4.1				
IV	Corn cobs	14.60	12.5	666	870	204	1.17
	Supplement (2/3 protein from Urea)	4.96	4.3				
V	Corn cobs	15.36	11.1	664	906	242	1.38
	Supplement (1/4 protein from fishmeal)	3.70	2.7				
VI	Corn cobs	16.09	11.9	662	899	237	1.36
	Supplement (Active Dry Yeast)	3.52	2.6				
VII	Corn cobs	16.14	12.0	669	905	236	1.35
	Supplement (Vitamin B_{12})	3.51	2.6				

Experiment IV

Growth data, feed consumption, and feed efficiency are summarized in table 5.

Corn cobs were successfully used as the sole source of roughage, when supplemented to make good their nutritional deficiencies, in the wintering ration of growing steers. In this experiment the daily gains ranged from 1.14 to 1.38 pound daily per steer. Although the differences are not mathematically significant, these data indicate that fish meal, active cell yeast or Vitamin B_{12} tend to contribute factors toward the growth of steers, being wintered on corn cobs, over and above that supplied in Purdue Cattle Supplement A or in the urea—substituted supplements.

Urea was used to supply from 1/3 to 2/3 (Lots II, III, and IV) of the protein equivalent for growing calves when additional energy was

supplied to compensate for the energy of protein supplement replaced by urea.

Experiment V

A detailed summary of the results of this trial is presented in table 6.

Gains in excess of two pounds per steer daily were made when either corn silage or grass silage was fed as the only source of roughage and when Purdue Cattle Supplement A was fed at the rate of 3.5 pounds per steer daily.

Corn cobs were superior to soybean straw for growing cattle during the winter.

Summary

Five experiments are reported in which ground corn cobs, oat straw, soybean straw, corn silage or grass silage were fed as the sole source of roughage to calves or yearling steers. Each steer received, in addition to the roughage being tested, a daily feed of 2.25 pound soybean meal, 1 pound molasses feed, minerals and a vitamin A and D concentrate (Purdue Cattle Supplement A).

When the individual roughages were fed with Purdue Cattle Supplement A, the following average daily gains were recorded: ground corn cobs, 1.28 to 1.56 pound; oat straw, 0.78 pound; soybean straw, 0.78 pound; corn silage, 2.06 to 2.21 pound; grass silage, 2.01 pound.

Some modifications of the original Purdue Cattle Supplement A were made for steers being fed corn cobs. When urea was used to replace from one-third to two-thirds of the protein equivalent supplied by soybean oil meal and this supplement was fed with ground corn cobs, no significant difference in growth rate was obtained when the gains of such fed animals were compared with those of steers receiving all their protein from soybean oil meal.

The addition of vitamin B_{12} or fish meal gave some apparent growth stimulation, although the difference was not significant.

Although the addition of live cell yeast gave an apparent growth stimulation, the addition of neither live cell yeast nor brewer's yeast resulted in a significantly increased growth rate.

The addition of two pounds of alfalfa meal—replacing two pounds of corn cobs—resulted in significantly increased growth.

Steers received no growth stimulation from the addition of distiller's dried solubles.

TABLE 6. SOYBEAN STRAW, CORN COBS, CORN SILAGE AND GRASS SILAGE
For Wintering Steer Calves
December 8, 1950–May 4, 1951—147 Days

Lot Ration	Steers Per lot	Av. Initial Wt., lb.	Av. Final Wt., lb.	Total Gain per Steer, lb.	Av. Daily Gain, lb.	Av. Daily Feed		Feed per lb. Gain	
						Roughage lb.	Supp. A lb.	Roughage lb.	Supp. A lb.
I Soybean Straw Supplement A	20	480	595	115	.78	13.30	3.50	17.1	4.5
II Corn Cobs Supplement A	20	478	698	220	1.50	13.37	3.50	8.9	2.3
III Corn Silage Supplement A	20	481	806	325	2.21	37.00	3.50	16.7	1.6
IV Grass Silage Supplement A	20	482	777	295	2.01	37.85	3.50	18.9	1.7

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MINERAL CONTENT OF CERTAIN CATTLE FEEDS USED IN NORTH CENTRAL KANSAS[1, 2]

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REPORTS on the mineral content of feeds usually are confined to only a few elements, and seldom do reports give data on content of both major and minor minerals. Moreover, there is a scarcity of data on the mineral content of certain forages and grasses. This investigation was undertaken to supply data on both major and minor elements of feeds important to the cattle industry of the Middle West.

Feed samples were taken from stocks used in typical beef cattle feeding operations at Kansas State College. However, the pelleted materials might at the present time be considered experimental insofar as beef production is concerned. All grasses, hays and silages were grown locally and other feeds were purchased on the open market. Although original sources of the latter were not always determinable, they conformed to normal supply situations.

Methods and Materials

This report contains data on feed samples collected over a period of about three years. Representative samples were collected at regular intervals and a composite sample was made of each feed from the same source that was used during the same period of feeding. The samples were ground in a small Wiley mill to pass through a 20-mesh screen. In order to minimize possible contaminations by grinding, silver-plated screens were used on those samples which were to be analyzed for minor elements.

All samples were dried in an oven at 103° C. and subsequently ashed overnight in an electric muffle at 625° C., unless otherwise indicated.

Sodium and potassium were determined by using the excitation unit of a Beckman Flame Photometer in connection with the large Littrow quartz spectrophotometer (Schrenk and Smith, 1950). Potassium was analyzed in a water solution representing 5 mg. of dry ash in 20 ml. of solution containing 1 mg. of lithium as an internal standard. Sodium

[1] This study was supported in part by funds contributed by Salt Manufacturers Association, Detroit, Michigan.

[2] Contribution No. 466, Department of Chemistry, and No. 188, Department of Animal Husbandry.

[3] Manhattan, Kansas.

was determined using 100 mg. of dry ash in 20 ml. of solution containing 0.15 mg. of lithium.

Calcium was determined by the same instrumental assembly according to the method described by Schrenk, Lentz and Glendening (1951). Since there was considerable variation in the calcium content, the amount of ash per sample was varied from 5 mg. to 40 mg. per 10 ml. of solution. Previous to dilution, the ashed samples were treated with 1:1 HCl to convert the calcium to a soluble form. Strontium was used as the internal standard. Data obtained on several check samples by the present procedure and the A.O.A.C. (1945) method were practically identical.

Magnesium, iron, copper and manganese were determined spectrographically, using the method developed by the Federal Soft Wheat Laboratory as described by Morris *et al.* (1945).

Colorimetric methods were used for phosphorus, cobalt and molybdenum. The phosphorus samples were wet ashed with H_2SO_4 and 30 percent H_2O_2 and taken up in Morgan's universal extracting solution. The color was developed by the use of ammonium molybdate and amino-naphthol-sulfonic-acid solution according to the method of Wolf (1944). Cobalt samples were wet-ashed with nitric and sulfuric acids; iron was removed by ether extraction; copper was removed by H_2S; and the color was developed by the use of a Nitroso-R salt solution (McNaught, 1942). Molybdenum was determined by the modified thiocyanate method on the dry-ashed materials (Ellis and Olson, 1951). All colorimetric readings were made on an Evelyn colorimeter with appropriate filter.

Chlorides were determined by the A.O.A.C. (1945) method, and soluble sulfates by the method of Woodman and Evans (1933).

Results and Discussion

Concentrations of various minerals in moisture-free samples of the several feeds analyzed are presented in table 1. The number of samples analyzed is shown in the parenthesis following the sample description. Original moisture of each feed is shown so that data may be recalculated to an "as-fed" or other basis if so desired, and protein content is given on a dry basis as an indication of the quality of feeds used.

Sodium, Potassium and Chloride

Sodium content of feeds varied from 0.002 to 0.08 percent. Where data for similar feeds are available these present values are somewhat

TABLE 1. MINERAL CONTENT OF FEEDS
(Moisture Free Basis)

	Original Moisture	Protein	Na	K	Cl	Ca	Mg	P	Fe	Sol. SO_4=	Cu	Co	Mn	Mo
	Percent										p.p.m.[1]			
Soybean Meal (6)[2]														
Av.	8.38	48.2	.018	1.88	.073	0.23	0.26	0.72	.016	.09	20.	.21	43.	2.4
Min.	7.58	47.3	.012	1.30	.010	0.10	0.20	0.64	.012	.02	13.	.19	35.	1.7
Max.	9.24	49.2	.024	2.32	.127	0.30	0.30	0.81	.023	.12	29.	.30	48.	3.3
Cottonseed Meal, Hydraulic Extracted (4)														
Av.	7.95	44.5	.016	1.11	.076	0.15	0.56	1.27	.017	.11	20.	.27	25.	3.7
Min.	7.40	44.0	.013	0.76	.046	0.05	0.37	1.20	.015	.10	13.	.18	18.	1.7
Max.	8.88	44.9	.020	1.71	.101	0.20	0.80	1.40	.020	.14	25.	.40	41.	5.0
Cottonseed Meal, Solvent Extracted (2)														
Av.	7.66	44.9	.010	1.03	.052	0.11	0.37	1.29	.015	.23	22.	.32	24.	4.1
Min.	7.63	44.8	.004	0.91	.051	0.11	0.35	1.25	.014	.12	21.	.29	24.	3.8
Max.	7.69	44.7	.016	1.15	.054	0.11	0.39	1.33	.016	.35	24.	.36	24.	4.5
Linseed Meal (1)														
	8.18	39.6	.060	0.92	.120	0.34	0.42	0.94	.019	.12	15.	.64	47.	—
Yellow Corn (5)														
Av.	10.94	10.6	.003	0.35	.050	0.01	0.10	0.32	.004	.02	4.	.23	9.	2.5
Min.	10.15	10.4	.002	0.32	.038	0.01	0.07	0.27	.003	.01	2.	.10	4.	0.7
Max.	11.64	10.8	.003	0.36	.056	0.02	0.12	0.39	.005	.05	6.	.30	14.	4.0
Milo Grain (1)														
	11.88	9.7	.008	0.38	.091	0.05	0.06	0.49	.002	—	14.	.14	16.	6.7
Dehydrated Alfalfa Pellets (3)														
Av.	7.57	18.2	.038	2.33	.259	1.56	0.36	0.35	.041	.31	34.	.25	52.	2.8
Min.	7.20	16.9	.036	2.15	.186	1.35	0.28	0.20	.028	.31	22.	.21	36.	1.8
Max.	8.20	18.8	.043	2.54	.400	1.70	0.45	0.48	.056	.32	53.	.30	63.	4.8

Alfalfa Hay, Composite of Several Samples (1)														
	8.99	16.3	.083	2.26	.444	1.32	0.45	0.43	.021	—	28.	.21	48.	0.9
Dehydrated Red Clover (2)														
Av.	7.76	17.2	.016	2.36	.104	1.03	0.44	0.27	.034	.03	17.	.22	39.	0.8
Min.	7.71	17.1	.016	2.18	.089	1.00	0.31	0.26	.030	.03	14.	.21	32.	0.8
Max.	7.82	17.3	.017	2.54	.120	1.06	0.57	0.28	.038	.08	21.	.23	47.	0.9
Dehydrated Brome Pellets (1)														
	8.29	17.8	.034	2.40	.256	0.54	0.20	0.27	.019	.14	22.	.55	50.	0.8
Silage, Corn (1)														
	74.10	8.5	.014	1.20	.151	0.54	0.57	0.27	.029	.14	55.	.14	78.	1.5
Silage, Atlas Sorghum (6)														
Av.	71.19	6.1	.017	1.10	.145	0.28	0.36	0.26	.029	.05	35.	.26	48.	0.9
Min.	65.00	4.2	.010	0.97	.070	0.21	0.28	0.17	.019	.01	22.	.13	41.	0.2
Max.	77.24	7.7	.033	1.40	.239	0.36	0.52	0.34	.034	.12	65.	.54	50.	1.8
Silage, Tennessee Orange (2)														
Av.	67.30	5.7	.016	1.05	.048	0.21	0.25	0.28	.023	.01	31.	.21	41.	1.4
Min.	65.00	5.3	.013	0.93	.047	0.21	0.18	0.28	.022	.01	29.	.12	40.	1.4
Max.	73.10	6.1	.020	1.17	.050	0.21	0.33	0.29	.024	.01	33.	.31	43.	1.4
Prairie Hay, Mostly Bluestem (8)														
Av.	7.04	5.5	.014	0.78	.136	0.32	0.30	0.15	.012	.03	25.	.17	53.	1.0
Min.	6.55	4.9	.003	0.50	.105	0.24	0.17	0.10	.005	.01	8.	.08	35.	0.2
Max.	7.62	6.0	.038	1.02	.164	0.36	0.41	0.23	.020	.07	51.	.32	61.	1.7
Prairie Grass, Mostly Dry Bluestem, Collected in Winter (2)														
Av.	7.19[3]	3.6	.008	0.20	.058	0.47	0.26	0.07	.040	.10	97.	.38	71.	0.6
Min.	6.97[3]	2.7	.008	0.09	.026	0.43	0.10	0.06	.034	.06	82.	.26	61.	0.4
Max.	7.42[3]	4.6	.008	0.30	.090	0.52	0.42	0.08	.047	.14	112.	.51	82.	0.8
Prairie Grass, Mostly Green Bluestem, Collected on Dates Shown (2)														
Apr. 19	8.58[3]	20.8	.028	1.51	.370	0.57	0.13	0.34	.047	.04	60.	.47	88.	2.4
July 17	8.66[3]	9.3	.014	0.36	.320	0.41	—	—	—	—	—	.48	—	1.1

[1] p.p.m. may be converted to percent by multiplying by 10^{-4}.
[2] Figures following feed names indicate number of samples analyzed.
[3] Moisture based on oven-dried samples.

smaller than those given for similar feeds in the older reports compiled by Black *et al.* (1939), Beeson (1941) and Morrison (1948). However, the values reported are similar to those published by Meyer *et al.* (1950) for feeds of similar types. Meyer *et al.* used the flame photometer in their sodium determination as did the present investigators. As pointed out by the Wisconsin workers, sodium values obtained by the flame photometer procedures frequently tend to be lower than those obtained by precipitation methods. Linseed meal, alfalfa hay and pellets, and brome pellets had the highest sodium contents while the other forages were relatively low. Corn grain contained the least sodium of all feeds tested.

Potassium content of all feeds was much greater than sodium, being in several cases approximately 100 times as great on a weight basis. Alfalfa, brome grass and clover had the highest potassium content. Potassium values obtained were similar to those given in the earlier compilations of Black *et al.* (1939), of Beeson (1941) and of Morrison (1948).

Chlorides in the feed usually were somewhat higher than sodium. On a molar basis, chloride concentrations frequently were more than twice the sodium concentrations, indicating that sodium chloride alone will not account for all chlorides present.

Calcium, Magnesium and Phosphorus

Alfalfa and clover contained more than one percent calcium; silages and grasses about one-fifth as much, and corn grain averaged .01 percent. In the concentrates, the magnesium content exceeded that of calcium, while the reverse was true for the legumes. Phosphorus exceeded the sum of calcium and magnesium in the concentrates, but was relatively lower in both the legume and non-legume forages. The data for calcium, magnesium and phosphorus are comparable to those in several reviews (Black, *et al.* 1939; Beeson, 1941, and Morrison, 1948).

Iron, Copper, Cobalt and Manganese

The iron content of feeds showed about a ten-fold range from 0.004 percent (40 p.p.m.) in corn to 0.047 percent (470 p.p.m.) in prairie grass. It has been very difficult to determine the iron requirement of cattle (Davis, 1951).

Copper was found to range from an average of 4 p.p.m. in corn to between 14 and 22 p.p.m. for other concentrates. Most of the forages

showed still higher values. Australian and New Zealand investigators, Bennetts *et al.* (1941) and Cunningham (1944) showed deleterious effects on cattle in regions where pasture plants contained less than 5 p.p.m. copper on a dry basis. On pastures which produced no deficiency symptoms in cattle a mean value of 11 p.p.m. of copper was given (Cunningham, 1950). The present data indicate that all mixed rations fed in the local investigation were well above the minimum for adequate nutrition as far as copper is concerned.

Milo grain and corn silage had the lowest cobalt contents, both being 0.14 p.p.m. on a dry basis. The highest cobalt content was found in linseed meal which analyzed 0.64 p.p.m. In recent years there has been much interest in the relation of cobalt to health of cattle and sheep. Wunsch (1937) pointed out that "Mortain Mains Disease" was a result of cobalt deficiency and Underwood and Harvey (1938) showed that the pasture plants in deficient areas in Australia contained from 0.02 to 0.07 p.p.m. cobalt while those from healthy areas averaged 0.16 p.p.m. Based on this literature and Beeson's (1950) conclusion that 0.07 p.p.m. may represent the minimum level for health in ruminants, the feeds investigated were adequate with respect to this element.

The grasses, hays and sorghums had the highest manganese content of the feeds analyzed, one sample of prairie grass containing 88 p.p.m. Corn grain with a mean value of 9 p.p.m. had the lowest manganese content of all the feeds tested. The manganese content of the mixed rations apparently was adequate, as the manganese requirements for cattle is probably below 10 p.p.m. (Davis, 1951).

It will be noted that higher concentrations of the heavy metals occur in grasses, hays and silages than in the concentrates. When considering these data one should not overlook the possibility of contamination by dust and soil in normal handling of these feeds. However, any such materials present in the feeds are probably consumed by the cattle.

Molybdenum

This element is of interest, first because apparently it is essential for plant growth (Monier-Williams, 1949), and second, because in certain regions of the world it is contained in plant materials in amounts toxic to animals. Ferguson, Lewis and Watson (1943) reported that the tert pastures of Somerset contained herbage of at least 20 p.p.m. molybdenum on a dry basis. More recently workers in the United States (Barshad, 1948 and Robinson and Edgington, 1948) have indi-

cated that there may be areas in this country where molybdenum toxicity may be manifested on pasture with a somewhat lower content of this element than the standard accepted by the British. The copper content of the ration may affect the molybdenum picture, as the two elements are reciprocally antagonistic in animal metabolism (Cunningham, 1950). None of the feeds investigated had molybdenum contents that could be interpreted as approaching toxic levels.

Soluble Sulfates

The amount of sulfur present as soluble sulfate was small. Alfalfa pellets had the highest content of soluble sulfate, about ten times that of red clover pellets. The sorghum silages and prairie hay samples were relatively low in content of soluble sulfates. The data on sulfates are of doubtful nutritional significance except to give a clearer picture of the ions present in the feed samples.

Summary

Several typical cattle feeds used in North Central Kansas were analyzed for mineral content and the results tabulated. Data are presented for the following constituents of the same feeds: sodium, potassium, chlorine, calcium, magnesium, phosphorus, iron, copper, cobalt, manganese, molybdenum and soluble sulfates. Based on requirements given in the recent literature, typical beef cattle rations composed of the feeds analyzed would not be deficient in copper, manganese, or cobalt, and molybdenum would not be present at toxic levels.

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ABSORPTION AND TISSUE DISTRIBUTION OF RADIOCALCIUM IN CATTLE[1]

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THE contributions of radiocalcium procedures towards an understanding of the metabolism of this element have been demonstrated in a number of studies with various species including rats (Singer *et al.* 1951, Harrison *et al.* 1950), dogs (Govaerts 1949), chicks (Migicovsky and Emslie 1950), hens (Spinks *et al.* 1949, Driggers and Comar 1949), goats (Aten and Heyn 1950), swine (Comar *et al.* 1952), and cattle (Hansard *et al.* 1951).

Studies with labeled calcium in large animals have been limited due to the following considerations: (a) availability and cost of isotopes, (b) cost of animals, as there will usually be no salvage value, (c) problems of radioassay of soft beta emitters, (d) management of the animals during and after administration of multimillicurie amounts of activity, and (e) disposal of activity-containing excretions and carcasses. An extensive program is underway in this laboratory designed to utilize radiocalcium in the study of mineral metabolism in cattle. The various phases under investigation include a study of absorption, physiology of bone formation, effects of age, role of calcium in the blood, placental transfer, calcification in the fetus, and others.

This paper presents some of the basic information obtained in regard to calcium and phosphorus analyses, and radiocalcium behavior for some 21 experimental animals. In this group of animals there were variations in age from seven to 36 months, differences in sex and stage of pregnancy, and three steers on varying calcium intakes. Examination of the data showed, however, that there were no significant differences due to these factors for the values presented in this paper. It is considered, therefore, that the data are representative of the normal animal in this age range.

Experimental

Hereford cattle were maintained from weanling calves under similar average farm conditions. They were placed in holding pens and, except

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where otherwise noted, fed nutritionally adequate rations—three to five months previous to and during the experimental period. The rations consisted of 4 to 6 pounds ground yellow corn and 0.5 pound wheat bran fortified with 1500 I.U. of Vitamin D in the form of irradiated yeast; water and Johnson Grass, Orchard grass, or Lespedeza roughage were fed *ad lib*. These cattle were consuming 16 to 23 gm. of calcium per day. Animals No. 233 and 232 were placed on a low calcium ration in which oat straw was used as a source of roughage. Animal No. 160 was supplemented to give an increase in calcium intake up to 30 gm. per day. All animals were consuming an average of 15 gm. of phosphorus per day and were generally in slight positive phosphorus balance.

Following the conditioning period, the animals were placed in metabolism units as described by Hansard (1951). Preliminary routine calcium and phosphorus balance trials were made, and after the animal had become adjusted to the stall and was behaving normally the radiocalcium solution was administered as a single dose either orally or intravenously (Hansard *et al.* 1951). Concurrent seven day chemical and radioisotope balance studies were started at the time of dosage. Detailed records of feed and weighback were kept and chemical analyses thereof were used to calculate the actual amounts of calcium and phosphorus ingested.

The calcium 45 was administered as a solution of the chloride which was adjusted to pH 6 immediately before use to eliminate any precipitation which might occur upon standing at this pH. Usually 2 to 3 mc. were used for the intravenous studies and about twice those amounts for oral administration. The mass of calcium administered varied depending upon the specific activity of the original preparation, but rarely exceeded 200 mg. per dosage. This amount was small compared to the amount normally ingested or present in the blood. Blood samples, as well as representative samples of feces and urine, were taken periodically during the 7-day experimental period. The slaughter procedure was as follows: The animal was stunned by a blow on the head and then suspended head down for complete bleeding from the jugular veins and carotid arteries; a blood sample was taken at this time. The tissues and organs were then removed with care being taken to avoid contamination with intestinal contents in animals that were dosed orally, and with blood in the cases of those animals dosed intravenously. Total weights of the various organs were recorded, and the contents of the various organs of the G. I. tract were removed and weighed

separately. Duplicate or triplicate 20 gm. samples of soft tissue and 10 gm. samples of bone were placed in tared porcelain crucibles and weighed immediately. The procedures were managed so as to eliminate any significant error due to loss of weight of fresh samples. Analyses for total calcium and radiocalcium were carried out as described by Comar *et al.* (1951) and those for phosphorus and ash by standard methods.

Results and Discussion

Absorption and Excretion of Oral Calcium: Table 1 presents the data from the series of total calcium and Ca^{45} balance studies. It may

TABLE 1. CALCIUM BALANCE AND EXCRETION OF ORALLY ADMINISTERED CA^{45} BY CATTLE

Animal No.	Sex	Age mo.	Weight lbs.	Percent Excreted		
				Total Ca in Feces	Ca^{45} in Feces	Ca^{45} in Urine
233	Steer	7	403	88	61	0.09
271	"	15½	581	87	73	0.01
190	"	26	763	82	59	0.02
203	"	36	1050	107	67	0.6
272	F. (3½ mo. preg.)	20½	614	68	78	0.07
290	F. (3½ mo. preg.)	23	690	107	71	0.09
264	F. (6 mo. preg.)	24	936	69	50	0.05
254	F. (6 mo. preg.)	25	873	73	55	0.2
151	F. (6 mo. preg.)	26	737	83	79	0.4
161	F. (8 mo. preg.)	29	854	42	62	0.1
80	F. (7½ mo. preg.)	32	811	126	77	0.1
				$85^1 \pm 17^1$	66 ± 8	$0.16 \pm .13$

[1] mean ± mean deviation

first be noted that the excretion in the urine was quite low, averaging 0.16 percent of the administered dose. The fecal excretion of radiocalcium was 66 percent ±8, indicating an average retention of 34 percent. The chemical calcium balance showed an average fecal excretion of 85 percent ±17, or a retention of only 15 percent. The results from chemical analysis showed a greater variation between individuals than did those based on activity measurements.

In general, the excretion of dietary calcium, as measured by calcium analysis, was higher than as measured by Ca^{45} assay. This can be interpreted to mean that some of the stable calcium in the feces, about 19 percent of the ingested calcium, was derived from sources other than the feed, presumably from the body stores. The question as to whether this endogenous calcium represents skeletal calcium which has merely been replaced or exchanged by Ca^{45} atoms, or whether it indicates an actual depletion of bone will be discussed in another paper.

TABLE 2. EXCRETION OF INTRAVENOUSLY ADMINISTERED Ca^{45} BY CATTLE

Animal No.	Sex	Age No.	Weight lbs.	Percent Excreted	
				Ca^{45} in feces	Ca^{45} in urine
232	Steer	12	505	16	0.23
243	Steer	19	760	12	0.02
304	F. (7 mo. preg.)	26	1076	17	0.54
162	Steer	36	1140	16	0.48
156	Steer	36	1157	14	0.15
				15 ± 2^1	$0.28 \pm .18$

[1] mean ± mean deviation

Absorption and Excretion of Intravenous Calcium: Table 2 presents values for the excretion, during the seven day balance period of intravenously administered Ca^{45}. It is shown that about 15 percent of the I. V. dose was excreted in the feces and 0.3 percent in the urine.

Figure 1 compares graphically the fecal excretion rate of orally and intravenously administered Ca^{45}. For clarity, values for two representa-

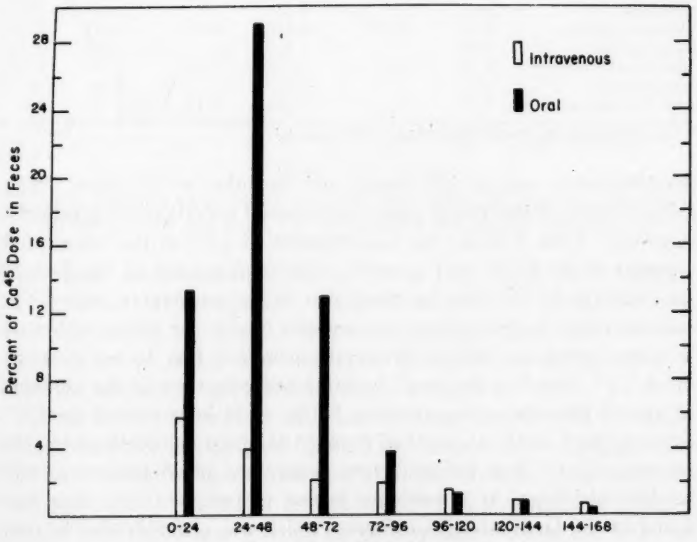

Figure 1. Fecal excretion rate of orally and intravenously administered Ca^{45}.

tive individuals are shown. The height of the bar indicates the percentage of the dose in the feces collected during the designated time interval. It is obvious that the peak of excretion after oral administration occurred in the 24 to 48 hour interval; intravenous calcium was excreted sooner, the peak occurring before 24 hours.

Secretion of Circulating Calcium into the G. I. Tract: In order to study the extent and rate of secretion of endogenous calcium into the lumen of the gastrointestinal tract, three yearling heifers were given labeled calcium intravenously. One was sacrificed ten minutes after

TABLE 3. SECRETION OF CIRCULATING CALCIUM^{45} INTO THE GASTROINTESTINAL TRACT OF CATTLE[1] (INTRAVENOUS ADMINISTRATION)

Time after Administration	10 min.		8 hours		30 hours	
	Percentage of dose $\times 10^3$ per gm.	Total percent of dose	Percentage of dose $\times 10^3$ per gm.	Total percent of dose	Percentage of dose $\times 10^3$ per gm.	Total percent of dose
Rumen	1.2		0.12		0.116	
Rumen contents	0.004	0.05	0.061	0.8	0.083	1.2
Omasum	—		0.13		0.059	
Omasum contents	0.045	0.09	0.035	0.09	0.121	0.33
Abomasum	0.81		0.18		0.070	
Abomasum contents	0.19	0.08	0.087	0.04	0.075	0.07
Small Intestine	0.89		0.26		0.089	
Small Intestine contents	0.24	0.46	0.088	0.21	0.130	0.37
Large Intestine	0.92		0.32		0.15	
Large Intestine contents	0.043	0.07	0.038	0.76	0.76	1.4

[1] Calculations standardized to body weight of 500 pounds.

administration, one at eight hours, and the other at 30 hours. These animals were subject to the same experimental conditions as previously described. Table 3 shows the concentration of Ca^{45} in the tissues and contents of the G. I. tract as well as the total percent of the dose in the contents. It may first be noted that in the ten minute animal the concentrations in the various tissues were about the same, with that in rumen being the highest. However, there was four to ten times as much Ca^{45} found in the small intestine contents than in the contents of any of the other compartments. In the eight hour animal the Ca^{45} concentrations of the tissues had dropped to about one-tenth of the ten minute values; these followed very closely the blood pattern as will be discussed later. At 8 hours the largest percentage of the dose was found in the large intestine contents, which was probably due in part to passage of the calcium from the small intestine and to absorption taking place from the small intestine. The next highest amount was present in the rumen contents, which may have been due to the saliva

and the increased opportunity for the rumen contents to come in contact with the rumen walls. This same trend was evident at 30 hours.

It is clear that in cattle, circulating calcium is secreted into all parts of the gastrointestinal tract, with the small intestines playing the major role. This is in general agreement with the observations of Wallace *et al.* (1951) on the excretion of Ca^{45} into the rat intestine.

Calcium 45 in the Blood: Figure 2 shows the amount of labeled calcium in the total blood of cattle as a function of time after oral or

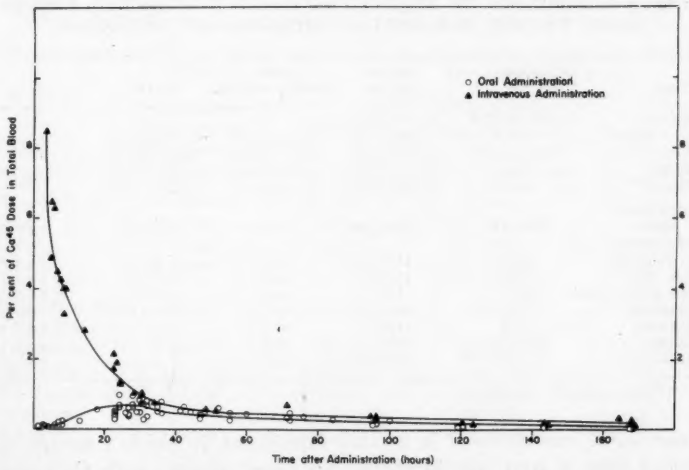

Figure 2. Calcium 45 in blood of cattle after oral and intravenous administration. (data are from 12 oral animals and seven intravenous)

intravenous administration of a single dose. Total blood was calculated as 7.7 percent of body weight. These data are from animals already described and of which there were 12 in the oral group and seven in the intravenous group. The individual points have been included to show the amount of spread which might be expected.

Let us first consider the rapid rate of disappearance from the blood of intravenously administered Ca^{45}. Five minutes after injection only about 30 percent of the dose was found in the blood and this continued to disappear at a rapid rate as indicated in figure 2, reaching about 3 percent at 8 hours and leveling off at less than 0.5 percent after 72 hours. As near as can be determined, there is a straight line disappearance between 100 percent at zero time and 3 percent at eight hours. It has already been shown that only about 15 percent of this

calcium is lost by excretion; this means that almost 85 percent of the dose administered finds its way to the skeleton of the animal. An estimation of what proportion of this Ca^{45} is merely exchanged with stable calcium of the skeleton, and how much is deposited as new bone must await further study.

This rapid disappearance cannot be considered as removal of excess calcium from the blood, since this pattern was observed in studies where less than 3 mg. of total calcium was administered. This same disappear-

TABLE 4. SUMMARY OF ANALYTICAL DATA ON SELECTED TISSUES FROM TWENTY-ONE CATTLE (MEAN±MEAN DEVIATION)

Tissue	Phosphorus mg./gm.	Calcium mg./gm.	Ash Percent fresh wt.	Ca/P	Ash/Ca
Plasma	0.059±0.003[1]	0.13 ±0.02	—	—	—
G. muscle	2.1 ±0.4	0.055±0.02	1.12±0.05	0.028±0.009	—
Liver	3.0 ±0.6	0.071±0.02	1.43±0.07	0.023±0.005	—
Kidney	2.5 ±0.4	0.12 ±0.03	1.15±0.07	0.052±0.016	—
Spleen	2.7 ±0.4	0.079±0.01	1.37±0.07	0.029±0.007	—
Metatarsus Shaft	105±13	210±19	56±4	2.0±0.2	2.2±0.2
Metatarsus Epiphysis	73±7	144±12	39±4	2.0±0.2	2.7±0.2
Rib Shaft	84±9	174±13	46±3	2.1±0.3	2.6±0.1
Rib External End	40±5	79±11	22±3	2.0±0.2	2.8±0.2
Mandible	82±9	163±15	43±4	2.0±0.2	2.7±0.2
Vertebra	61±8	110±17	35±5	2.0±0.1	2.7±0.1
Molars	119±13	247±15	63±13	2.1±0.2	2.6±0.1
Incisors	126±11	233±15	64±3	1.9±0.1	2.8±0.1

[1] Plasma inorganic phosphorus

ance curve was observed in another experiment in which a sample of blood from a steer was incubated for fiteen minutes with Ca^{45} and then injected back into the animal. It seems clear that circulating calcium atoms are continuously being rapidly removed from the blood and are replaced by calcium atoms absorbed from the tract and by those exchanged out of the skeleton itself.

Figure 2 also shows the rate of appearance in the blood of orally administered calcium 45. The largest amount found in the blood averaged about 0.6 percent with a peak at around 30 hours after administration. After this time the oral and intravenous curves were very similar. It must be remembered that as the Ca^{45} atoms are absorbed from the tract into the blood they will probably be removed from the blood at the same rate as shown for the Ca^{45} introduced directly into the blood by intravenous injection. The curve indicates that the greater portion of dietary calcium was absorbed within 30 hours which is in agreement with the excretion data already presented.

Analytical Results: Table 4 presents a summary of analytical data

on selected tissues from twenty of the cattle already described. It was of particular interest to note the constancy in bone of the ratios of Ca to P and of ash to Ca. It is concluded that under the conditions of this experiment the ash values can be used to calculate adequately the calcium and phosphorus content of bone.

Tissue Distribution of Ca^{45}: Table 5 presents a summary of the distribution of Ca^{45} in selected tissue seven days after oral and intravenous administration. Concentration values are expressed as percent

TABLE 5. SUMMARY OF DISTRIBUTION OF CA^{45} IN CATTLE AT 7 DAYS AFTER ADMINISTRATION[1] (MEAN±MEAN DEVIATION)

	Oral (13 animals)		Intravenous (5 animals)	
Tissue	Percentage of dose×10^5 per gm. fresh weight	Specific[2] Activity	Percentage of dose×10^5 per gm. fresh weight	Specific Activity
Plasma	0.86±0.3	7.6±2	1.9±0.6	18±4
G. Muscle	0.43±0.1	8.1±3	1.1±0.1	22±4
Liver	0.43±0.1	6.0±2	1.6±0.5	21±4
Kidney	0.80±0.2	7.2±2	2.8±0.5	18±6
Spleen	0.57±0.2	7.3±2	1.6±0.5	22±6
Metatarsus shaft	68±25	0.32±0.1	236±74	1.1±0.3
Metatarsus epiphysis	93±33	0.69±0.2	363±110	2.5±0.7
Rib Shaft	120±30	0.67±0.2	479±130	3.0±0.9
Rib Sternal End	314±87	4.0 ±2	955±120	13 ±2
Mandible	235±94	1.4 ±0.6	700±90	4.4±0.4
Vertebra	238±90	1.9 ±0.7	765±205	5.7±1
Molars	45±15	0.19± .07	50[3]	0.2[3]
Incisors	36±15	0.16± .07	88±26	0.4± .09

[1] Calculations standardized to body weight of 500 pounds.
[2] Specific activity expressed as percent of dose×10^5 per mg. of calcium.
[3] Values available from only 1 animal.

of the dose x 10^5 per gm. fresh weight, and specific activity values are expressed as percent of the dose x 10^5 per mg. of calcium present in the fresh tissue.

Excretion data already presented indicated that retention of Ca^{45} averaged 34 percent and 85 percent for oral and intravenous administration, respectively. This is reflected in the Ca^{45} content of the tissues, as it will be noted from table 5 that the intravenous values ranged from two to four times the corresponding oral values.

In considering the soft tissues it should be noted that circulating Ca^{45} soon comes to equilibrium between the soft tissues and the plasma. For example, in the animals reported in table 3, the specific activity

of the plasma was about three times that of the G. muscle ten minutes after injection, whereas these specific activities were equal in the eight hour animal. In a series of pigs receiving Ca^{45} by injection in this laboratory the specific activities of plasma and muscle were found to be equal in the animals sacrificed at one hour or longer after administration. It is also noted from table 5 that there were no significant differences between the specific activities of the various soft tissues; in two animals it was found that there were no significant differences between the specific activities of the following additional soft tissues: thyroid, thymus, parotid gland, mandibular gland, intestinal lymph nodes, adrenals, penis, pancreas, gall bladder, bladder, eye, lung, trachea, tongue and esophagus. This can be interpreted to mean that calcium ions in the blood are rapidy interchangeable with the calcium ions in the soft tissues mentioned.

Before consideration of the Ca^{45} values for the skeletal tissues it may be helpful to visualize the deposition. Figure 3 shows an autoradiogram of the metatarsus from Steer No. 243. This was prepared by the method of Lotz *et al.* (1952) which consisted essentially of sectioning the whole bone with a band saw and exposing a 2mm. thick section on No-screen X-ray film. The dark areas represent Ca^{45} deposition. It is obvious that the heaviest laydown was directly below the epiphyseal plate at the distal end, and that there was well-defined accumulation directly beneath the periosteum. In parts of the compact shaft there was very little laydown, except for discrete longitudinal streaks which probably represent the Haversian systems. There was a spotty distribution in regions of trabecular bone. These autoradiograms emphasize the difficulties involved in obtaining representative samples of bone for radioassay.

Referring to table 5 it may be noted that the bones were one to several hundred times higher in Ca^{45} than were the soft tissues. However, the specific activities of the bones were much lower than those of the soft tissues. This means that the calcium of the bones is not completely exchangeable with the calcium of the plasma, and is in agreement with in vitro studies in this laboratory which have indicated that only about 20 percent of bone calcium is exchangeable.

The accumulation of calcium appeared to parallel metabolic activity as may be noted from the specific activity values. The sternal end of the rib showed the highest turnover with a specific activity almost approaching that of the soft tissues. Next were the vertebra and the mandible, respectively. The shaft of the rib and the epiphysis of the

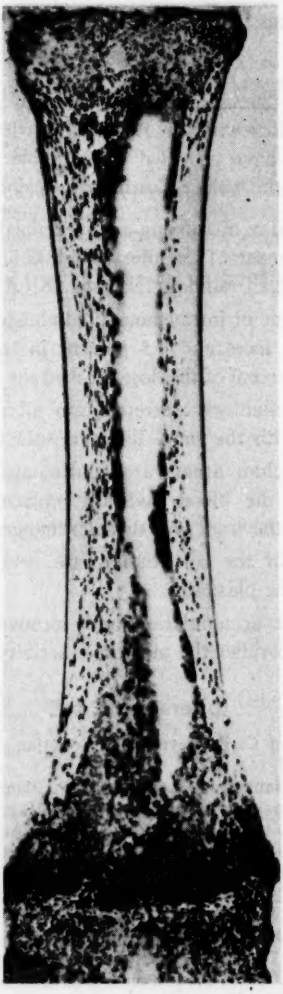

Figure 3. Autoradiogram of metatarsus from Steer No. 243. Note heavy laydown of Ca^{45} beneath epiphyseal plate at distal end, well defined accumulation directly beneath the periosteum, discrete longitudinal streaks representing the Haversian systems in the compact bone of the shaft, and the spotty distribution in the regions of trabecular bone.

metatarsus had values which were close together and about twice those of the metatarsus shaft. As expected, the teeth showed the lowest turnover.

Summary

Radiocalcium studies with 21 Hereford cattle in the age range of seven to 36 months have provided considerable basic data which are presented and from which the following conclusions were drawn:

1. The fecal excretion of calcium as determined by chemical analysis was 85 ± 17 percent of the dietary intake; the fecal excretion of orally administered radiocalcium was 66 ± 8 percent.
2. About 15 percent of intravenously administered radiocalcium was excreted in the feces and 0.3 percent in the urine which means that about 85 percent of the dose reached the skeleton.
3. Circulating calcium was secreted into all parts of the gastrointestinal tract with the small intestines playing a major role.
4. Circulating calcium atoms are continuously and rapidly being removed from the blood and are replaced by calcium atoms absorbed from the tract and those exchanged out of the skeleton.
5. Calcium ions in the soft tissues are generally interchangeable with those in the plasma.
6. In general, the accumulation and turnover of calcium in the various bones parallel the metabolic activity.

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TECHNIQUES EMPLOYED IN CULTURAL INVESTIGATIONS OF THE BACTERIOLOGY OF BOVINE RUMEN CONTENTS[1]

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KNOWLEDGE of the essential role played by specific bacterial species of the bovine rumen is relatively scant. It has been pointed out that cultural studies have certain limitations. Baker's (1945) objections are based on an admitted difficulty, namely, that all physiological types of bacteria present in the rumen will not grow on a given medium. This fact should serve as an incentive to more intensive investigations in this direction. In addition, bacteria so obtained are not seen in relation to their natural environment. Nevertheless, it would appear axiomatic that cultural studies constitute the essential mode of attack in determining the physiological role of rumen bacteria. The advances made by Elsden (1946), Hungate (1950) and Johns (1951) were brought about in this manner.

The purpose of this paper is to present data resulting from a critical examination of some of the cultural techniques published in studies of rumen contents. This was found necessary since difficulty had been encountered with these methods, some of which appeared to have no sound basis for recommendation and others which seemed overly complicated. Results based on improvements and modifications are therefore presented. It is possible to apply these results to a systematic cultural investigation of rumen bacteria.

Experimental

Rumen samples were obtained from Ayrshire, Holstein, and Jersey heifers approximately one year old. Diets consisted of U.S. No. 2 leafy alfalfa hay, a commercial concentrate mixture containing 16 percent protein and limited grazing on blue grass pasture.

Sample Collection

Samples were obtained by an esophageal tube 5 feet in length (7/16″ inside diameter). The technique recommended by Gall *et al.* (1949) was repeatedly attempted with little success. Solid masses of hay, grass, etc., clogged up the tube, or else acted as filters so that only very

[1] This investigation was supported in part by a grant from the Ralph E. Ogden Foundation. Paper No. A-368, Contribution No. 2357 of the Maryland Agricultural Experiment Station.

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fluid samples were obtained. Samples obtained by hand insertion of the tube into the rumen followed by gentle up and down motion[3] and without resort to hand pumps or other vacuum systems were always easily obtained. The sample (1,000–1,500 ml.) was collected in a 2 liter Erlenmeyer flask equipped with a glass funnel. The flask was stoppered and returned to the laboratory for processing.

Media preparation

The medium recommended by Gall *et al.* (1947) was unsatisfactory due to the formation of a bulky white precipitate. The medium of Hungate (1950) was used with excellent results. This medium consists of 20 ml. of an inorganic salt solution (containing ammonium, chloride, calcium, magnesium, sodium, and phosphate ions), a reducing agent (0.02 percent sodium thioglycolate), 15 ml. of filtered rumen fluid, 0.0001 percent resazurin as an oxidation-reduction indicator, and agar. Sterilization is carried out under carbon dioxide after which sterile bicarbonate (0.25 percent) is added aseptically with further carbon dioxide gassing to give a pH of 7. Minor additions to this medium were made as follows: 0.05 percent sodium thioglycolate, 0.075 percent cystine, and 0.2 percent each of glucose and cellobiose. The latter two compounds were Seitz-filtered in proper concentrations and added after autoclaving the medium along with sufficient sodium carbonate[4] and cysteine hydrochloride to give final concentrations of 0.3 percent and 0.02 percent respectively. This medium was designated as #1 and was prepared in 100 ml. lots.

A second medium used primarily to avoid the necessity of using rumen fluid consisted of the following: Eugon agar (B.B.L.), plus Hungate's (1950) salt solution, plus 0.05 percent sodium thioglycolate and 0.0001 percent resazurin, prepared in the same fashion as medium #1. After sterilization Seitz-filtered cellobiose, sodium carbonate and cysteine hydrochloride were added to give final concentrations as above. This medium is very clear and was designated as #2.

Dilution Blanks

In order to process rumen samples under controlled anaerobic conditions dilution blanks were devised which were light orange when anaerobic and bright pink when exposed to trace amounts of oxygen.

[3] The authors are indebted to Dr. W. D. Pounden of the Ohio Agriculture Experiment Station, Wooster, Ohio, and Dr. B. C. Hatziolas of the University of Maryland, Dairy Department, for this technique.

[4] The authors are indebted to Mr. Marvin Bryant, U.S.D.A. Bureau of Dairy Industry, Beltsville, Maryland, for this suggestion.

In addition Tween 80[5] was incorporated to disperse bacteria from food particles and clumps. The blanks were prepared on the same day they were to be used and were sterilized at 121° C. for 20 min. in rubber stoppered square milk dilution bottles. The stoppers were held in place by means of added weights. The blanks were gassed with oxygen-free[6] carbon dioxide for 2 minutes immediately before using. The composition of the blanks was as follows: $(NH_4)_2SO_4$, 0.05 gm.; KH_2PO_4, 0.02 gm.; K_2HPO_4, 0.05 gm.; $CaCl_2$, 0.005 gm.; $MgSO_4$, 0.005 gm.; NaCl, 0.1 gm.; $NaHCO_3$, 0.4 gm.; cystine, 0.075 gm.; sodium thioglycolate, 0.05 gm.; dextrose, 0.5 gm.; resazurin, 0.0001 gm.; Tween 80, 0.01 gm.; distilled water to 100 ml.

Processing Procedure

The sample was mixed by stirring in the Erlenmeyer flask and 10 gm. of the well-mixed sample added from a sterile graduate to a gassed 90 ml. dilution blank. The sample was shaken on a machine (3″ stroke, 294 excursions per min.) for 2 minutes. Serial 1 ml. transfers were made in the same fashion using gassed 99 ml. blanks to 10^{-9} of the original sample. Dilutions 10^{-7} to 10^{-9} were added to 6 ml. of melted, cooled, media #1 and #2 contained in rubber stoppered (125×16 mm.) test tubes. The samples were mixed in the agar under carbon-dioxide and then the agar was solidified in a thin film on the walls of the tube by rotating under a stream of cold water (Hungate, 1950). The tubes were incubated for 48 hours at 39° C. At the end of this time well-isolated colonies were picked and serially diluted (by loop) through three more tubes of melted agar. After 48 hours at 39° C. well-isolated colonies were picked to slants and used for further study. Colonies were selected for purification only from the 10^{-8} or 10^{-9} dilutions. Occasionally a colony from 10^{-7} was selected because of some unusual property. Pure cultures were stored in the refrigerator and transferred semi-monthly (Bryant, 1951).

Experiment 1. In order to determine the effect of the time interval between collection and processing on the total bacteria count of rumen contents samples were collected and processed within 15 minutes using medium #1. The sample was then allowed to stand at room temperature (21° C.) and counts were made at various intervals up to eight hours.

Experiment 2. In an effort to determine the necessity of keeping the

[5] Tween 80 is a commercial designation for a polyoxyethylene derivative of sorbitan monooleate.

[6] Tank carbon dioxide was passed through Fieser's solution. This was prepared as follows: 20 gm. potassium hydroxide were dissolved in 100 ml. water. Two gm. sodium anthroquinone β-sulfonate and 15 gm. of sodium hydrosulfite were then added to this solution. It was stirred until a clear blood red color develops. After it was saturated with oxygen the color changed to a dull brown. The amount above will absorb 788 ml. of oxygen. (Fieser, 1941).

sample under anaerobic conditions at all times prior to processing the following experiment was performed: Immediately after processing, a sample was divided into two 50 ml. portions; one was placed in a stoppered 250 ml. Erlenmeyer flask under carbon-dioxide, the other was shaken vigorously in an unstoppered flask at 15–30 minute intervals. Counts were made at intervals up to four hours. Medium #1 was used for this work.

TABLE 1. EFFECT OF TIME INTERVAL BETWEEN COLLECTION AND PROCESSING ON TOTAL COUNTS

Sample	Hours Interval	Count $\times 10^7$
1	0	71
	4	79
	8	82
2	0	22
	3	38
	5	24
3	0	28
	5	20
4	0	12
	4	21
5	0	30
	4	28
6[1]	0	22
	4	18
7[1]	0	19
	4	22
8	0	30
	4	42

[1] Samples 6 and 7 were the same samples processed twice.

Experiment 3. Since rumen fluid added to a medium makes it opaque and further since it is of variable composition a comparison between the total counts obtained with media #1 and #2 was made. Since medium #2 was devised for nutritionally fastidious bacteria it was hoped to omit rumen fluid from further consideration. In addition, microscopic examinations of the flora growing upon these media were made.

Results and Discussion

Experiment 1. The results of this experiment are shown in table 1. It should be noted that even when the interval between collection and

processing was eight hours there was little difference in the total count of the sample. This finding suggests that samples can be collected from distant farms and be processed at the laboratory some time later. The results obtained here are at variance with the recommendations of (Gall *et al.*, 1947, 1949).

The conclusion to be drawn from this experiment is that the bacterial *status quo* in rumen samples is maintained numerically for some time. Either the bacteria in the sample are inhibited but not killed or else the growth and death processes are in equilibrium. It should be pointed out that duplicate runs on the same sample result in counts which are quite close (see samples 6 and 7).

TABLE 2. EFFECT OF AERATION ON TOTAL COUNTS

Sample	Hours Interval	(Count $\times 10^7$) Not Shaken	Shaken
1	0	12	—
	4	21	11
2	0	30	—
	4	28	27
3	0	30	—
	4	42	37

Experiment 2. From the results presented in table 2 it is evident that shaking in air did not affect markedly the total counts. It would seem that special precautions (Gall *et al.*, 1947) to exclude air from the rumen sample are unnecessary. It is likely that the redox potential in the rumen sample can be maintained for some time by reducing substances contained therein. For example, rumen fluid reduces resazurin quite rapidly when preparing medium #1. There is no *a priori* reason for believing that rumen bacteria are killed immediately upon contact with air. The studies of Pounden and Hibbs (1950) seem to support this view. These investigators noted that calves may receive rumen bacteria by ingesting feeds contaminated with organisms from older animals. These organisms are undoubtedly exposed to air for various lengths of time prior to ingestion. It should be emphasized that previous experiments wherein anaerobic conditions were maintained immediately upon collection of the sample until processing showed little differences in total counts from the results obtained with the collection method described here.

Experiment 3. In only four instances did medium #2 give total counts higher than #1 (table 3). There is a spread between the counts which is much greater than that expected from variations in the method. The flora which appeared on each medium was found to be essentially the same. This was determined by examining microscopically, stained (crystal violet) preparations from all of the colonies which appeared on the 10^{-8} and 10^{-9} dilutions in each medium. The average colony size after 48 hours' incubation was always larger on medium #1 than on #2. The only advantage of medium #2 was its transparency.

TABLE 3. COMPARISON OF TOTAL COUNTS WITH MEDIA #1 AND #2

Medium #1				Medium #2			
Sample	Count $\times 10^7$	Sample	Count $\times 10^7$	Sample	Count $\times 10^7$	Sample	Count $\times 10^7$
1	10	11	11	1	12	11	8
2	84	12	30	2	22	12	6
3	26	13	27	3	22	13	12
4	22	14	23	4	33	14	11
5	38	15	19	5	39	15	12
6	24	16	18	6	51	16	8
7	28	17	23	7	6	17	7
8	20	18	30	8	4	18	16
9	12	19	42	9	7	19	14
10	21	20	37	10	14	20	17

Colonies were very easily counted in this medium. Growth in stabs of pure cultures was also easily seen. In figures 1 and 2[7] are shown electron micrographs of shadowed preparations of two of the predominant organisms found in this work. Colonies of these organisms are usually present in the 10^{-8} or 10^{-9} dilutions. In figure 1 is shown a diplococcus and in figure 2 a long thin rod-shaped bacterium. The diplococcus is similar to that described by Johns (1951) but larger and more numerous. Gall and Huhtanen (1951) also have isolated an organism morphologically similar to this. The criteria of Eldsen and Phillipson (1948) for a true rumen organism have been thus partially met. It remains to be shown whether these organisms bring about a chemical transformation known to occur in the rumen.

The data suggest that there are factors in rumen fluid necessary for the optimal growth of rumen bacteria. This conclusion seems warranted in view of the fact that Eugon agar is recommended for nutritionally

[7] Major Reginald L. Reagan of the Livestock Sanitary Service Laboratory, Maryland State Board of Agriculture, University of Maryland prepared these micrographs.

fastidious organisms of the usual saprophytic and parasitic type. The factors in rumen fluid are as yet unknown although work is now being done on the problem in this laboratory.

It should be noted that the total counts obtained in this investigation are somewhat lower than those reported on by Gall *et al.* (1949). This

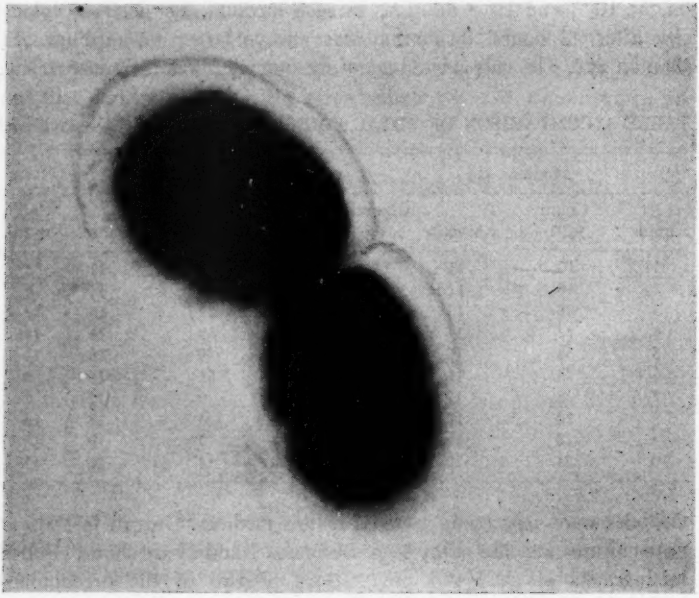

Figure 1. Electron micrograph of a 24 hour culture (medium #1) of a bacterium isolated frequently from rumen samples. Chromium shadowed at tangent 2/8, magnification 25,000 X.

is perhaps due to the fact that these workers used the solid materials obtained by straining the rumen fluid whereas the samples reported on here were processed without alteration.

Summary

Data are presented which indicate that the total bacteria counts obtained on rumen samples vary only slightly when held at room temperature for time intervals up to eight hours before processing.

Aeration of rumen samples by vigorous agitation over periods of

time up to four hours results in little change in the total bacteria count.

Rumen fluid contains unknown substances essential for optimal growth of some rumen bacteria. These substances were not provided

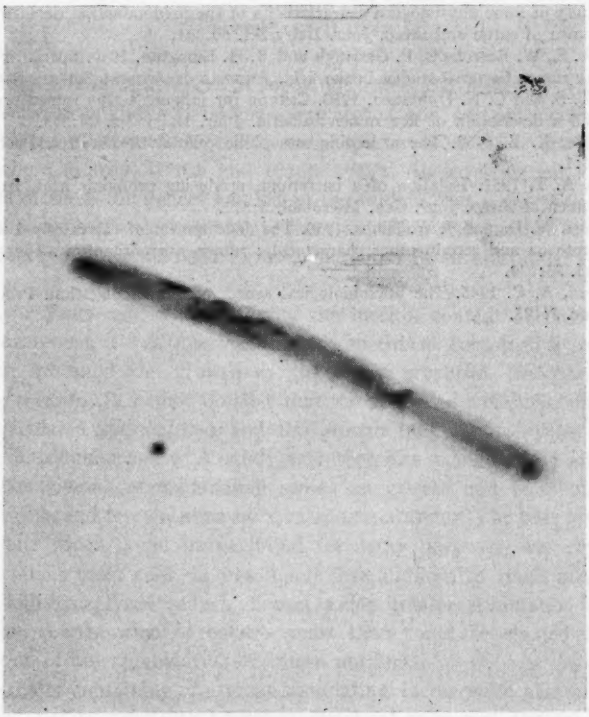

Figure 2. Electron micrograph of a 24 hour culture (medium #1) of a bacterium isolated frequently from rumen samples. Chromium shadowed at tangent 2/8, magnification 25,000 X.

by a commercial medium devised for nutritionally fastidious bacteria. Modifications of techniques used in processing rumen samples are suggested in the light of the findings presented.

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THE NATURE OF A YEAST-STIMULATING AGENT IN MAMMALIAN SPERMATOZOA[1]

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THE striking differences of metabolic rates and metabolic efficiency between epididymal bovine spermatozoa and certain specimens of ejaculated spermatozoa of the same species have been summarized elsewhere (Lardy, Ghosh and Plaut, 1949). An investigation into the cause of these differences has led to the finding that extracts of semen can be prepared which stimulate the respiration and aerobic glycolysis of epididymal spermatozoa to rates characteristic of ejaculated spermatozoa.

Some years ago, when studying the inositol content of bovine and human semen, it was observed that the specimens contained a principle which inhibited the growth of the assay organism, *Saccharomyces carlsbergensis*. It seemed possible that the decreased metabolic efficiency of ejaculated spermatozoa and the growth inhibition of yeast might have a common cause. A study was therefore made of the effect of the components of mammalian semen on growth and metabolism of yeast cells and fermentation by zymase preparations. The best response, and one which could be exploited for assay purposes, was obtained with intact yeast cells. It was found that addition of small quantities of alkali-hydrolyzed semen effected a far greater stimulation of the aerobic fermentation of baker's yeast than could be elicited by the addition of any combination of known nutrients.

Extracts containing the yeast-stimulating factor were also effective in uncoupling oxidative phosphorylation (Lardy and Ghosh, 1949; Johnson and Lardy, 1950) and it was assumed that a single substance was responsible for the effects in spermatozoa, in yeast and in rat-liver preparations. Similar activity was found in mild alkali-treated testicular tissue but not in a variety of other tissues tested. The yeast-active factor

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[2] We are indebted to the Department of Dairy Husbandry, University of Wisconsin, the Badger Breeders Cooperative and the Wisconsin Scientific Breeding Institute for generous supplies of bull semen. Prof. L. E. Casida kindly provided the boar, ram and rabbit semen from his experimental animals.

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has been isolated in crystalline form from carbon tetrachloride extract of alkali-hydrolyzed hog testis tissues previously defatted with acetone, and has been identified as elemental sulfur. As little as 0.4 microgram of elemental sulfur per ml. of fermentation medium stimulated the aerobic fermentation to anaerobic level without *apparently* affecting respiration and thus completely inhibited the Pasteur effect.

It must be emphasized that, unlike the extracts, elemental sulfur failed to show any activity in inhibiting Pasteur effect in bovine epididymal spermatozoa or in uncoupling oxidative phosphorylations in washed liver residues or mitochondrial preparations. The nature of the agents responsible for these activities will be discussed in subsequent papers. In this paper evidence is provided for the occurrence of a substance in spermatozoa which, upon contact of these cells with seminal fluid, is liberated in a water-soluble, non-dialyzable form. The latter on mild alkaline hydrolysis yields the lipid-soluble yeast-stimulating factor which has now been identified as elemental sulfur. A following paper (Ghosh, Plaut, and Lardy, 1952) deals with the effects of semen fractions on epididymal spermatozoa, and a later paper will deal specifically with the relation of sulfhydryl compounds to rates of metabolism of epididymal spermatozoa.

Material and Methods

Semen was collected by means of an artificial vagina from dairy bulls in regular breeding service. Experiments with fresh semen were begun within ten minutes of collection. For large scale preparation purposes, semen was accumulated in the frozen state. Epididymal spermatozoa were obtained as described previously (Lardy *et al.*, 1945)

Fresh, foil-wrapped baker's yeast (Red Star Brand) was employed in the fermentation studies which were carried out at pH 4.6 and at 30° C. Respiration and fermentation were measured manometrically and the results expressed as Q values (cmm. of gas exchange per mg. dry yeast per hour). For anaerobic fermentation the gas phase was 95 percent N_2 and 5 percent CO_2. The fermentation medium contained the following ingredients (all expressed in grams): acetic acid, 1.50; sodium acetate, 2.05; KH_2PO_4, 1.36; $MgSO_4 \cdot 7H_2O$, 0.32; $MnSO_4$, 0.10; glucose, 7.90; thiamin, 0.10; pyridoxine, 0.05; niacin, 0.05; calcium pantothenate, 0.05; water to make 500 ml. (pH 4.6). Its composition was suggested by Prof. M. J. Johnson. Each flask contained 1.5 ml. of the above medium, 1.0 ml. of a 0.5 percent yeast suspension

(1.5 mg. dry wt.) in water and 0.5 ml. water. Additions of semen or other materials replaced an equal volume of water. After an equilibration period of ten minutes readings were taken at 15 minute intervals and the Q values were calculated for the successive hourly intervals. Respiration and fermentation results were consistently repeatable within 5 percent.

Results and Discussion

Evidence for a specific stimulatory agent from semen: Preliminary experiments showed that the rate of aerobic fermentation of yeast in

TABLE 1. YEAST FERMENTATION ASSAY OF SEMEN FROM DIFFERENT BULLS FOR THE ACTIVE PRINCIPLE

(0.3 ml. of boiled semen were employed in each assay)

Bull No.	Q_{CO_2} Control	+Semen	Percent Stimulation of fermentation
1	181	265	47
2	191	264	38
3	135	251	86
4	174	296	70
5	164	263	60
6	159	252	58
6 (ashed)[1]	159	169	6

[1] Ash from semen was dissolved in dil. HCl and neutralized before addition of a quantity equivalent to 0.3 ml. semen.

the test system employed could be increased, up to a maximum of 30 to 86 percent above the control rate, with increasing quantity of boiled bull semen (up to 0.5 ml. per flask). The maximum response varied from sample to sample. In general, stored semen, after being frozen and thawed several times, gave a better response. The respiration and anaerobic fermentation were not, however, affected to any significant extent by semen. The aerobic fermentation system was employed as the standard assay procdeure for the determination of the activity of samples.

Table 1 shows the stimulatory activity of semen specimens from six different bulls. The active principle was thought to be organic in nature because an equivalent amount of semen ash dissolved in dil. HCl and neutralized to pH 4.6 was completely inactive (table 1). It was possible that the nitrogenous material present in boiled whole

semen was responsible for the increase in frementation rate above the control. However, boiled extracts of other animal tissues gave only slight stimulation and, furthermore, when a variety of amino acids, asparagine and ammonium salts were tested at different concentrations the greatest increase in aerobic CO_2 production during the first hour was only 35 percent greater than the control. These nitrogenous nutrients increased initial fermentation only slightly; their effect was progressive indicating that growth was proceeding at an appreciable rate. Since much greater responses were obtained with semen, and since the effect was noticeable from the very beginning of the experiment, the presence of a specific stimulatory agent in semen was indicated and the problem thus warranted investigation.

To determine the nature and stability of the active principle semen samples, fresh or stored, were subjected to mild hydrolysis with dilute (0.17 M) HCl and NaOH respectively for a period of ten minutes on a boiling water bath. After immediate cooling and neutralizing to about pH 5, the samples were assayed for activity (percent stimulation of aerobic fermentation of yeast over the control in the first hour). Acid hydrolysis caused partial loss of the activity while alkaline hydrolysis increased the activity greatly as shown in figure 1. This remarkable increase in the rate of aerobic fermentation by semen was considered to result from a "stimulating agent" and not from nutrient material for the reasons presented above and especially because the stimulation of fermentation obtained with alkali-hydrolyzed semen was apparent from the beginning of the experiment.

Again, respiration and anaerobic fermentation were not enhanced appreciably by the stimulatory agent. When the active material was later obtained in purified form it was found not to increase respiration or anaerobic fermentation of the yeast.

To determine whether alkaline hydrolysis liberated a similar stimulatory substance from other natural materials, comparison experiments were made with tissues of animal, plant and microbial origin. Only comparatively large amounts of these substances could bring about any measurable increases in the rate of fermentation. The stimulation was rather insignificant at first and increased with time (figure 2). The rate of respiration also progressively increased indicating again that the nitrogenous material was supporting yeast growth. Furthermore, alkaline hydrolysis enhanced only slightly the stimulating activity of the other materials. Extensive experiments which demonstrated that the marked stimulation of fermentation by alkali-hydrolyzed semen

was due to some specific substance present in semen, have been documented elsewhere (Ghosh, 1949).

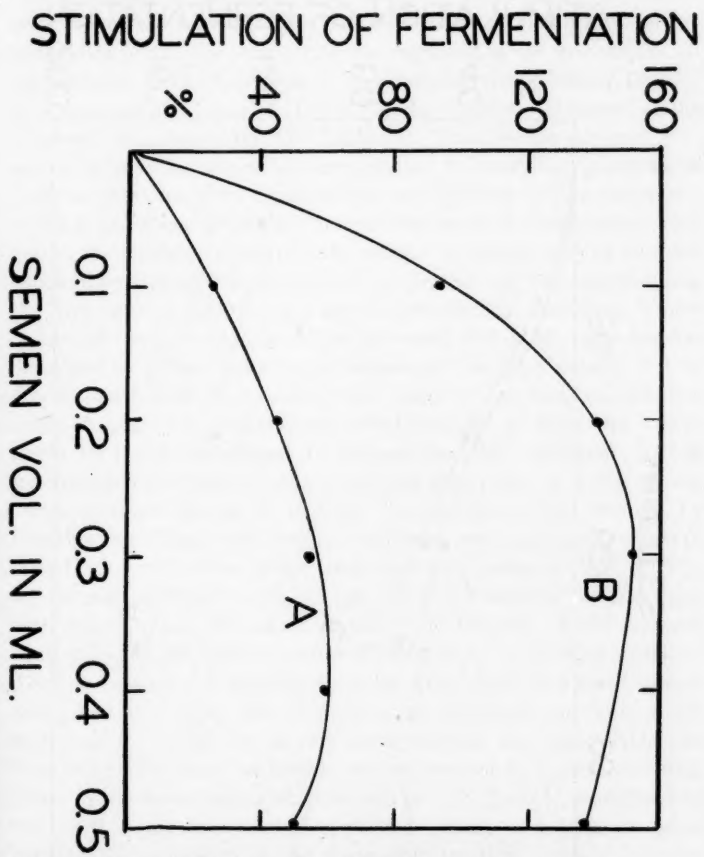

Figure 1. Effect of boiled and alkali-hydrolyzed semen on aerobic fermentation of yeast. A. Boiled Semen. B. Alkali-hydrolyzed Semen.

In an attempt to identify the active principle with known substances, the effects of a variety of compounds on yeast fermentation were studied under the standard conditions. The substances tested, in addition to the amino acids already mentioned, included boiled yeast, nucleic acids and lecithin before and after alkaline hydrolysis, inositol, biotin, pyrexin,

spermine, several higher fatty acids and esters and also some of the steroid hormones. None showed activity similar to hydrolyzed semen.

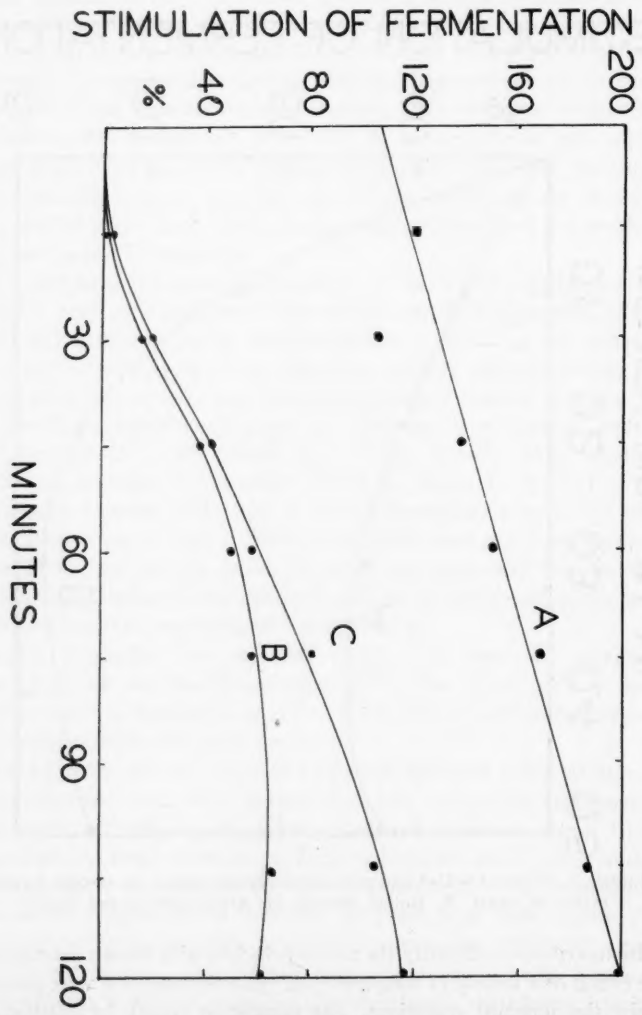

Figure 2. Stimulation of yeast fermentation by alkali-hydrolyzed semen, alfalfa and liver. A. Alkali-hydrolyzed semen (0.2 ml.). B. Alkali-hydrolyzed alfalfa (20 mg. dry wt.). C. Alkali-hydrolyzed liver (20 mg. dry wt.).

The above observations led to the tentative conclusion that a novel yeast-stimulating substance can be extracted from semen but not from certain other normal tissues.

Fractionation of semen and testis tissue for preparing active concentrates. Since alkaline hydrolysis had been shown to enhance the yeast-stimulating activity of semen, this was employed as the first step in all fractionation work. Experiments to determine the optimum time of hydrolysis indicated that, in 0.17 N NaOH, maximum liberation of the substance occurred in ten to 15 minutes. The response of yeast, *i.e.*, percent stimulation of aerobic fermentation, to increasing quantities of crude preparations of the regulator was roughly linear up to a maximum; addition of excess quantities inhibited the rate of fermentation. One unit of the regulator was arbitrarily defined as the quantity of material which stimulated aerobic fermentation 50 percent over the control during the first hour in the standard test system already described. A procedure for concentrating the active substance with good recoveries was developed as follows. Semen was treated with one-fifth volume of 1 N NaOH and heated on a boiling water bath for ten minutes with frequent shaking. The solution was cooled; brought to about pH 5 with dilute HCl and centrifuged to remove insoluble materials. To the supernatant fluid three volumes of acetone were added in a thin stream during constant stirring or shaking. The precipitate was removed by filtration; the filtrate and acetone washings were combined and evaporated to a small volume at low temperature and pressure. After acidifying the solution with 1 N HCl to pH 2–3, it was extracted several times with diethyl ether. Extraction at pH 7 effected only partial recovery while at higher pH there was little or no recovery in the ether solution.[4] The ether extract was evaporated at low temperature to a small volume and stored at —5° C. The preparation at this stage was fairly stable and could be stored for several weeks without any appreciable loss in activity. For assay the extract was evaporated to dryness *in vacuo*, taken up in water with a drop or two of 1 N NaOH, neutralized to about pH 5 and then diluted to desired volume with water to get a homogenous suspension of the active lipid fraction.

The activities and percentage recovery at each stage in a typical fractionation according to the above procedure are shown in table 2. The Roman numeral designations will be used subsequently to identify fractions at these various stages of purification.

[4] However, extraction of the ether solution containing the active principle with dilute $NaHCO_3$ or dilute NaOH did not displace the substance into the aqueous phase. Since the substance was not an acid, it was assumed that the original inability to extract it with ether from the neutral or alkaline solution was due to the presence of soaps.

The above fractionation procedure was employed for preparing active concentrates of the regulator for the purpose of studying its effect on the metabolism of yeast and spermatozoa and also for further purification. Figure 3 shows the typical dose-response curve for such a partially purified preparation of the regulator containing 0.1 to 0.2 mg. of dry matter per unit of activity. Concentrates of the "regulator" were also prepared from bull and hog testes. The frozen testis tissue was

TABLE 2. FRACTIONATION OF ALKALI-HYDROLYZED BULL SEMEN WITH ACETONE AND ETHER

All assays at a level equivalent to 0.2 ml. whole semen

	Sample assayed	Q CO_2	Percent Stimulation of fermentation	Active factor units (from 50 ml. semen)	Percent Recovery[1]
I	None (control)	157			
II	Alkali-hydrolyzed semen	316	101	505	
III	75 percent acetone soluble	300	90	450	89
IV	Residue from above	173	10	50	10
V	Ether extract (at pH 2) from III	274	74	370	73
VI	Residue from above	159	1	5	1

[1] From alkali-hydrolyzed semen.

pulverized in the cold and thoroughly extracted with acetone at 0°. From four to five parts of acetone by weight were used for each extraction and a total of three to four extractions were required to free the tissue of moisture and fat. After the final extraction the residue was dried *in vacuo*. The fat-free residue was then suspended in water (100 ml. per 10 gm. dry testis powder), 1/5th volume of 1 N NaOH was added and the hydrolysis and subsequent fractionation carried out as described above.

Solvent Distribution. A sample of ether extract (stage V) from hog testis was subjected to counter current distribution by the technique of Craig, 1944, Craig and Post, 1949. The solvent system was prepared by equilibrating 90 percent ethanol with a sufficient volume of skelly-solve A (low boiling petroleum ether) to make the final volume of each of the two phases equal. A 24-plate separation was made and the skelly A fractions in different tubes were assayed in triplicate. The

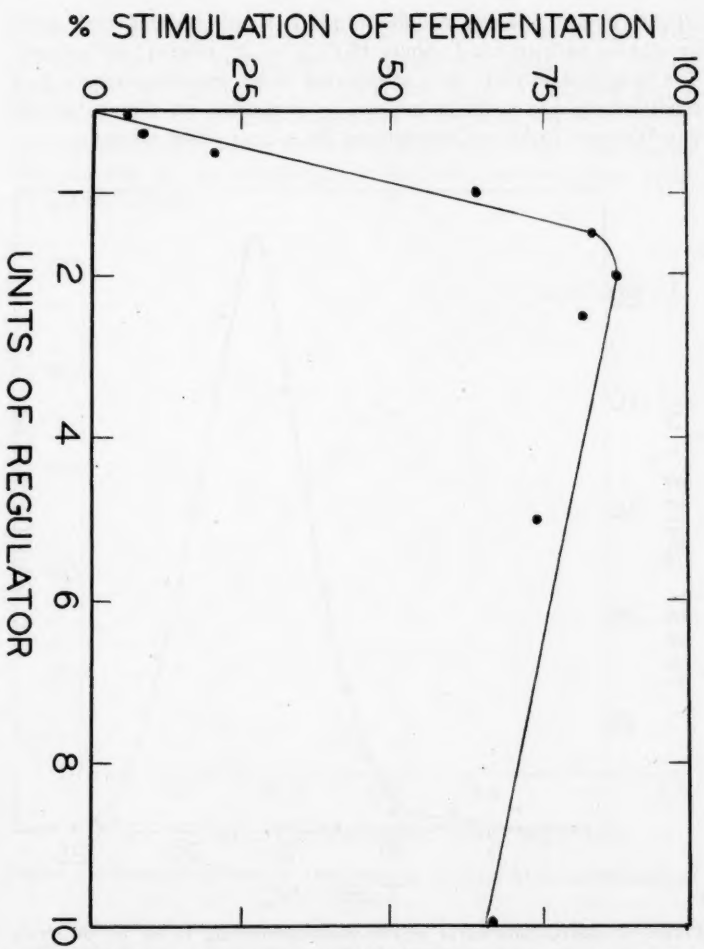

Figure 3. Typical dose-response curve for a partially purified preparation of the metabolic regulator.

yeast-stimulating activity was found to be distributed in a symmetrical peak (figure 4), indicating that a single active component was present.

Isolation and identification of elemental sulfur. The extraction procedure was shortened by the use of carbon tetrachloride. The cooled

alkaline hydrolysate was acidified to pH 2–3 and extracted three times by shaking with an equal volume of CCl_4 (C. P. reagent; sulfur-free). The combined extracts were evaporated under reduced pressure to a small volume and could be assayed by measuring an aliquot directly into Warburg flasks and evaporating the solvent under vacuum.

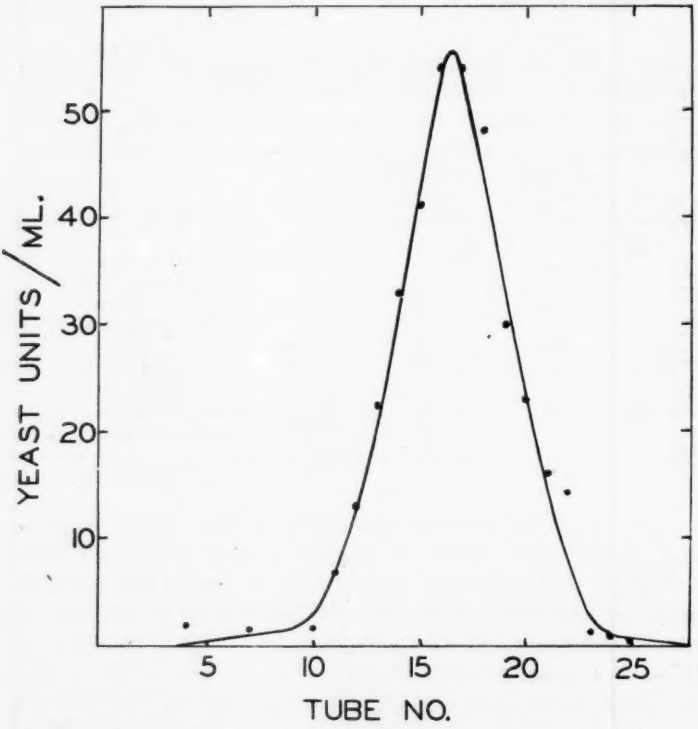

Figure 4. Distribution curve of the yeast-stimulating factor in the Craig counter-current separation.

The carbon tetrachloride extraction procedure was also successfully employed for preparing highly active concentrates from bull semen acetone powder. The carbon tetrachloride extract contained one unit of activity per 4.5 micrograms of dry matter. This represented a purification of more than 1000 fold when calculated on the basis of dry matter in the original alkali-hydrolyzed semen.

On keeping concentrated CCl_4 extracts from hog testis at —5° C

for several days, pale yellow crystals separated. The crystals were carefully collected, washed free from adhering lipids with absolute alcohol and then redissolved in CCl_4 for assay. A response of one unit of activity (50 percent stimulation of fermentation over control) was obtained with less than one microgram of dry matter. The residual lipid material in the mother liquor had comparatively little yeast-stimulating activity.

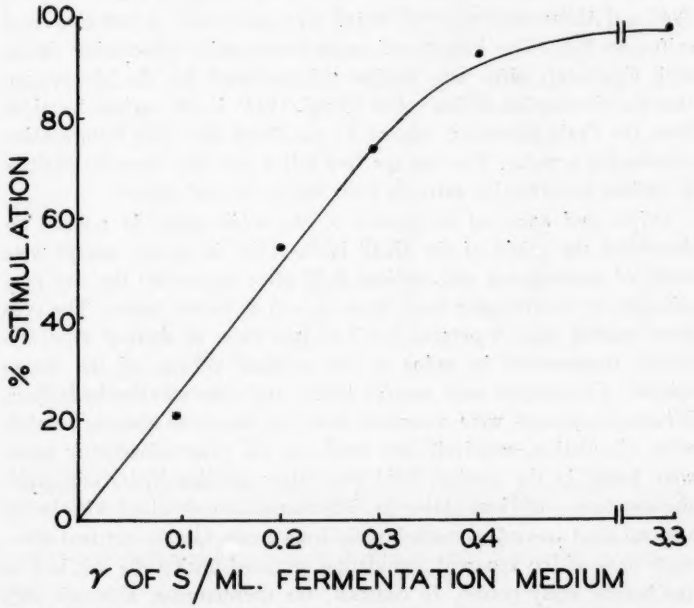

Figure 5. Stimulation of aerobic fermentation of yeast by elemental sulfur.

On microscopic examination the crystals were found to be monoclinc needles which broke down into small rhombic crystals on recrystallization with a drop of carbon tetrachloride on the microscopic slide. The rhombic crystals were identified as elemental sulfur by melting point (110–111° C; rhombic sulfur, m.p. 112° C) and from immediate formation of black mercuric sulfide when rubbed with mercury in a mortar. The identity was verified by reproducing the stimulating effect on yeast fermentation with pure rhombic sulfur dissolved in sulfur-free carbon tetrachloride. Similar quantities of colloidal sulfur in water were as effective as crystalline sulfur. Figure 5 shows the assay curve

using microgram quantities of elemental sulfur. The response was practically linear up to a miximum just as was observed with purified extracts from semen or testis. Respiration and anaerobic fermentation remained unaffected at these concentrations.

It is somewhat surprising that the fermentation-stimulating effect of elemental sulfur has not been accorded more interest before. Negelein (1925) had shown that 10^{-4} M H_2S stops respiration and consequently stimulates aerobic fermentation of yeast, and von Euler and coworkers, 1944 and Ahlstrom *et al.*, 1945, found that yeast could reduce elemental sulfur to H_2S. The identity of yeast-fermentation-stimulating factor with elemental sulfur was further substantiated by the observation that the distribution of free sulfur (Feigl, 1946) in the various fractions from the Craig separation (figure 4) paralleled the yeast fermentation stimulating activity. The test for free sulfur was also strongly positive in carbon tetrachloride extracts from bull testis and semen.

Origin and mode of occurrence of the labile-sulfur in semen. To determine the origin of the alkali labile-sulfur in semen, assays were made of spermatozoa and seminal fluid after separating the two constituents by centrifuging from fresh as well as stored semen. The cells were washed with .9 percent NaCl to free them of seminal fluid and finally resuspended in saline to the original volume of the semen aliquot. The samples were assayed before and after alkaline hydrolysis. When spermatozoa were separated from the semen as soon as possible after ejaculation, relatively low levels of the yeast-stimulating agent were found in the seminal fluid even after alkaline hydrolysis under the standard conditions (table 3). The stimulation obtained with boiled seminal fluid seemed to result largely from a non-specific nutrient effect since most of the apparent stimulation occurred during the last half of the hourly assay period. In contrast, the spermatozoa, although they comprise a relatively small fraction of the semen volume, contained greater quantities of the alkali-liberated yeast stimulating agent. Epididymal spermatozoa likewise are a rich source of this substance (expt. 5, table 3; *cf.* Lardy *et al.*, 1949). There was a distinct and progressive increase in the amount of the stimulating agent which could be released by hydrolysis of seminal fluid with increasing time of incubation of the whole semen. This increase did not occur when fresh or boiled samples of seminal fluid (from which all spermatozoa had been removed) were incubated for similar time intervals.

An azoospermic ejaculate was devoid of significant activity even after alkaline hydrolysis.

It seems likely that the small amount of alkali-hydrolyzable yeast-active substance occurring in the seminal fluid at the time the earliest separation could be made (15 minutes after ejaculation in experiments 1 and 3, table 3) had been liberated from the spermatozoa.

TABLE 3. LIBERATION OF THE YEAST-STIMULATING AGENT AND ITS PRECURSOR DURING STORAGE OF SEMEN AT ROOM TEMPERATURE

		% Stimulation of fermentation[1] by			
	Hrs.	Spermatozoa		Seminal fluid	
Expt.	incubated	Boiled	Alk. hyd.	Boiled	Alk.hyd.
1.	¼	9	—	12	32
	1	7	60	10	35
	6	10	87	12	61
	30	8	106	16	81
2.	½	6	81	27	41
	5½	5	89	20	84
3.	¼	7	128	25	51
	24	18	136	33	90
4.	1	26	100	33	46
	5½	24	100	28	112
5.	Epididymal spermatozoa	4	82		
6.	Azoospermic semen			17	28[2]

[1] In expts. 1, 2, 3 and 6 the level of assay was equivalent to 0.1 ml. of whole semen, in expt. 4 of the level was 0.2 ml. In expt. 5 the samples assayed contained 1.8×10^8 epididymal spermatozoa.

[2] Larger quantities of this specimen were extracted with solvents (see text) to separate the fermentation-stimulating agent from non-specific nutrients. An aliquot of this extract equivalent to 0.2 ml. of semen gave 11 percent stimulation of aerobic fermentation.

The above results indicate that the yeast-stimulating principle occurs in the spermatozoa in a bound form which, even after boiling, is unavailable to the yeast cell. From this bound form it is progressively liberated into the seminal fluid in the form of a water soluble complex which is still inactive as far as acceleration of yeast fermentation is concerned. When aged semen was centrifuged at high speed to separate all spermatozoa and the supernatant fluid dialyzed, with constant stirring, for 48 hours, none of the alkali-labile precursor was found in the dialysate but was recovered, nearly quantitatively, in the cellophane sack.

It appears, from the results of experiment 1, table 3, where sub-

maximum levels were tested, that the original bound form in spermatozoa is more resistant to alkaline hydrolysis than it is after a period of storage in semen.

The question next arose as to what biochemical processes were involved in the liberation of the water soluble complex from the spermatozoa. Enzymes of the seminal fluid seem to be involved for it was found that live spermatozoa, washed to free them of seminal fluid, and stored in a buffered salts-glucose medium for 24 hours at room temperature, did not contribute any of the activating material to the

TABLE 4. ACTIVITY OF SEMEN FROM DIFFERENT SPECIES ON AEROBIC FERMENTATION OF YEAST

Species	Semen ml.	% Stimulation of fermentation	
		Boiled	Alk. hyd.
Human	0.04	—	15
	0.08	—	40
	0.40	112	132
Ram	0.07	—	104
	0.30	30	106
	0.50	—	106
Boar	0.15	26	124
Rabbit	0.20	36	100

medium as determined by assay of the fluid both before and after alkaline hydrolysis. The significance of the sulfur-containing substance liberated from spermatozoa by seminal enzymes will be discussed in later papers.

Occurrence of the regulator in semen of other species. Semen from various other species; namely; rabbit, ram, boar and man showed similar stimulating activity on yeast fermentation (table 4). Alkaline hydrolysis considerably increased the activity of the semen samples. It will be noticed that the human semen sample showed very good activity even in the unhydrolyzed state. In several other human specimens the activity was appreciably enhanced by alkaline hydrolysis.

Summary

A search for the cause of differences in the metabolic characteristics between bovine spermatozoa obtained from ejaculated semen and those collected from excised epididymides has led to the finding that extracts

of semen stimulate respiration and glycolysis of epididymal spermatozoa and fermentation of yeast.

A method has been described for the biological assay of the yeast-stimulating substance.

The yeast-stimulating substance can be obtained from semen of the bull, ram, boar, rabbit, and man. It can also be obtained from testis tissue but not, by the same procedures, from a variety of other tissues.

On the basis of yeast assay the regulator seems to occur in a bound inactive form in epididymal or freshly ejaculated spermatozoa from which, under the influence of seminal fluid, it is progressively liberated into the medium, presumably in a combination still inactive for yeast cells. The yeast-active component can be liberated by mild alkaline hydrolysis of spermatozoa or whole semen.

A method has been described for preparing partially purified concentrates of regulator from semen suitable for studying its biochemical significance. The yeast-active principle has been isolated in crystalline form from hog testis extract and identified as elemental sulfur.

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SERUM ALKALINE PHOSPHATASE ACTIVITY IN COWS AND EWES ON WINTER WHEAT PASTURE

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THE determination of serum alkaline phosphatase activity has been used as a diagnostic and prognostic procedure in various pathological conditions (Bodansky, 1933, Yaguda 1936, and Roe and Whitmore 1938). There are a few reports in the literature of serum phosphatase activity in both laboratory and farm animals. Madsen *et al.*, 1947, report a decreased phosphatase activity along with a lowered blood calcium and inorganic phosphorus in beef cattle affected with anasarca due to a vitamin A deficiency. Hibbs *et al.*, 1945, observed in rachitic Guernsey calves, increased serum phosphatase activity which decreased as the blood calcium and inorganic phosphorus were increased in treatment. Young and Underdahl (1948) determined the serum alkaline phosphatase in suckling pigs up to 50 days of age. They found a decrease in activity with age. However, considerable variation occurred as was indicated by coefficients of variation of 23–91 percent. The serum phosphatase and phosphorus in normal and starved rabbits have been studied by Jørgensen (1944), who reported variations from animal to animal and from time to time. Both substances were reduced in starvation. During a 29-day experimental period, albino rats on a magnesium deficient diet showed a rapid decrease in serum magnesium and phosphatase (Snyder and Tweedy, 1942).

We have been attempting to establish the levels of some serum components of both Hereford cattle and Rambouillet sheep being used in investigations on wheat pasture grazing conducted at the PanTech Field laboratory by the Texas Agricultural Experiment Station, Texas Technological College and the Bureau of Animal Industry, U. S. D. A. This paper reports our observations of the serum alkaline phosphatase activity in Hereford cows and Rambouillet ewes while grazing winter wheat.

Experimental Procedure

Serum samples were taken every 28 days from a group of 40 Hereford cows and ten Rambouillet ewes from late in October, 1950, through

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early in April, 1951. The animals were part of the PanTech Farms herd and were supplied by Texas Technological College for our use. The cows were from three to five years old and had been bred to calve in November, 1950. The ewes were yearlings that had been bred to lamb in November, 1950. All calves and lambs were dropped by the time of the December samples. However, all but two of the lambs were lost during an extremely sudden cold wave shortly after the December sheep samples were taken. During the experimental period the cows were handled in one group and all received the same care and treatment. They were given free access to a pasture of winter wheat. The only supplement was salt, free choice. The sheep were handled in a similar manner. All animals were in good health throughout the experiment. The initial serum samples were taken when the animals were moved from summer range grass pasture to winter wheat pasture late in October and early in November.

The blood was drawn directly into centrifuge tubes and the serum was separated by centrifugation as soon as the clot was formed. The alkaline phosphatase was then determined with the fresh serum by a modification of the procedures of Fiske and Subbarow (1925) and Shinowara *et al.* (1942) and essentially as outlined by Hawk *et al.* (1949). Several other serum components including calcium and inorganic phosphorus were determined also.

Results and Discussion

The serum phosphatase findings for the cattle are given in table 1 which show an extremely wide variation between animals. The greatest difference between samples on a given date was 45.6 Bodansky units (March, 1951) and variations of 20 units were not uncommon. However, the level remained relatively constant for each animal, the higher levels showing wider fluctuations. In general, a cow with a high initial activity remained high on subsequent samplings, and conversely, those animals having a low level initially remained low.

The activity levels, in addition to extending over a wide range, did not follow a normal distribution curve. Approximately 44 percent of the values found were between 1.0 and 2.9 Bodansky units, yet almost 18 percent lay between 13.0 and 24.9 units. Because of the wide variation it was not deemed advisable to suggest a normal range for alkaline phosphatase activity for cows grazing winter wheat.

Serum calcium and inorganic phosphorus levels determined from the same serum samples had mean values of 11.0 ± 0.04 mg. percent and

TABLE 1. ALKALINE PHOSPHATASE LEVEL, IN BODANSKY UNITS, IN SERUM OF CATTLE

Animal No.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
1	1.6	2.4	1.7		2.0	
2[1]	0.9	1.9			2.1	
3	1.4	1.3		1.5	1.4	
4	2.4	1.6		4.6	1.4	
5	3.6	3.5			4.5	4.2
6	19.4	13.3			20.3	19.2
7	19.3	11.7	15.8		24.3	20.7
8	2.9	3.2	2.4		3.0	
9	1.9	7.0		7.1	4.5	2.8
10[1]	10.8	5.5			19.6	17.0
11	2.4	3.4			3.0	
12	22.1	8.4			17.6	
13	1.4	1.8	7.4		3.2	
14[1]	1.4	—		5.3	1.5	
15	5.6	6.0			5.9	5.8
16	9.8	4.8			14.4	11.8
17[1]	6.6	7.0			7.6	6.9
18	7.2	4.8			5.7	
19	2.0	3.3	4.2	7.3	4.8	3.9
20[1]	4.3	5.4		8.9	4.8	5.2
21	1.5	2.1			2.7	2.3
22	2.5	2.2	2.8		2.9	4.9
23[1]	5.0	—			4.2	5.6
24	16.1	10.7	1.3		16.0	16.5
25	1.8	3.4		3.7	3.0	2.3
26	2.1	2.9			2.4	
27	1.1	—		2.6	1.3	
28	0.8	0.8	0.8		1.0	
29	2.1	2.9			—	
30[1]	11.9	13.6			19.7	
31	2.3	8.0	2.8		3.2	2.6
32	2.3	2.7			2.4	1.9
33[1]	1.7	2.0			7.3	2.0
34	2.1	4.0		2.4	2.3	
35	2.3	2.1			2.4	
36	—	2.9			2.1	
37[1]	18.1	21.0			46.6	
38	1.9	2.1			2.9	
39[1]	1.1	1.6			1.8	1.8
40	14.3	20.0	24.5	30.3	24.6	31.0

[1] Did not have calves.

5.4±0.05 mg. percent, respectively, with standard deviations of 0.66± .03 and 1.47±0.05. The coefficients of variation were 5.97±0.23 and 27.3±1.1. There did not appear to be any relationship between the

serum calcium or inorganic phosphorus levels and the variations observed in the alkaline phosphatase activity. The coefficients of correlation between the alkaline phosphatase activity and the serum calcium level and the alkaline phosphatase activity and the serum inorganic phosphorus were +0.120 and −0.197 respectively.

The extreme variations and wide range of activity found in the cattle were in sharp contrast to the relatively narrow normal range for the serum alkaline phosphatase activity in man (Hawk *et al.*, 1949) and rabbits (Jørgensen, 1944). However, a wide variation in pigs has

TABLE 2. ALKALINE PHOSPHATASE LEVEL, IN BODANSKY UNITS, IN SERUM OF SHEEP

Animal No.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
301[1]	9.0	18.7	13.8	10.0	15.9	14.8
302	7.6	10.5	10.5	16.0	10.8	11.3
303	16.3	16.7	12.2	17.2[2]	11.5	13.3
304	7.1	12.1	5.1	5.2	9.3	6.3
305	7.8	12.5	10.7	10.2[2]	8.3	9.1
306	13.0	14.1	8.6	19.5[2]	12.7	21.5
307[1]	5.1	6.2	9.0	12.0	10.0	10.5
308	7.0	9.7	9.5	12.6[2]	12.6	11.9
309	8.0	12.7	9.5	12.0[2]	9.6	5.4
310	7.9	7.0	8.3	9.0[2]	9.8	12.8
Average	8.9	12.0	9.7	12.5	11.1	11.7

[1] Did not have lambs.
[2] Lost lambs during cold wave.

been reported (Young and Underdahl, 1948). It is now generally accepted that an elevation in serum alkaline phosphatase is indicative of some condition involving either liver damage or bone metabolism. The level in the healthy animal is influenced markedly by age and to some extent by diet (Moog, 1946). However, we have observed a wide variation between apparently healthy adult cows that had received identical treatment. It appears that more samples on cattle would be highly desirable.

The data on phosphatase activity in sheep are given in table 2. Sheep did not show as wide a variation as cattle but a greater variation than either man or rabbits. The individual variation in serum alkaline phosphatase activity appeared to be greater in sheep than in cattle, but the variation from animal to animal is much less. The maximum variation between animals was 16.1 Bodansky units (March sample). The mean value of the 60 samples was 11.1±0.3 Bodansky units and the

median, 10.5 ± 0.4. The standard deviation was 3.51 ± 0.22 and the coefficient of variation, 31.7 ± 0.2. A relatively narrow spread of values was found when the averages for each bleeding date were compared. All values were within 2 Bodansky units of the mean of all samples. The level of activity appeared to be slightly higher when the ewes were grazed on winter wheat than on summer range grass pasture. The close agreement of the mean and median was indicative of a fairly normal distribution of values and that a normal range of values of 7.5 to 14.5 Bodansky units might be suggested for alkaline phosphatase activity in sheep.

Serum calcium and inorganic phosphorus determinations on the same sera had means of 11.26 ± 0.5 mg. percent and 5.27 ± 0.13 mg. percent with standard deviations of 0.57 ± 0.04 and 1.47 ± 0.09, respectively. The coefficients of variation were 5.06 ± 0.31 and 27.9 ± 1.9. Again there does not appear to be any definite relation between the variation in alkaline phosphatase activity and serum calcium and inorganic phosphorus levels. The coefficient of correlation between the alkaline phosphatase activity and the serum calcium level was $+0.150$, and between the alkaline phosphatase and inorganic phosphorus it was -0.023.

It should be noted that all determinations were made on apparently healthy cows and ewes, and the calcium and phosphorus levels were within "normal" limits. An entirely different picture might be present in pathological conditions and during treatment and recovery. No explanation other than an insufficient number of samples is readily available for the wide variation in serum alkaline phosphatase levels in apparently healthy adult cows receiving the same diet and treatment.

Summary

Determination of the serum alkaline phosphatase activity in 155 samples from 40 Hereford cows over a period of six months revealed a wide variation in activity between animals. The level remained relatively constant for an individual animal. Because of the extreme variation observed, no attempt was made to suggest a normal range of serum values in cows.

Serum alkaline phosphatase level in sheep as determined from 60 samples taken from a group of ten Rambouillet ewes over a six month period does not vary as widely from animal to animal as it does in cattle. However, the variability from time to time for the same animal was greater.

The values found were mean, 11.1 ± 0.3 Bodansky units; median 10.5 ± 0.4; standard deviation, 3.51 ± 0.22; and coefficient of variation, 31.7 ± 0.2. The close agreement of the data suggests a normal range of 7.5 to 14.5 Bodansky units for sheep while grazing on winter wheat.

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AUREOMYCIN SUPPLEMENTS IN LAMB FATTENING RATIONS[1]

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DURING recent years, aureomycin supplements have been found to increase the rate of gain and reduce scours in young pigs. Carpenter (1950); Catron *et al.* (1951), Cunha *et al.* (1950); Jukes *et al.* (1950).

Rusoff (1951) fed a ration containing 2 percent of Lederle's A.P.F. at a level to provide 90–180 mg. of aureomycin per calf daily. Increased rate of gain and more thrifty appearance were evident in calves receiving aureomycin as compared with the control group. Bartley *et al.* (1950) fed a fermentation product containing residual aureomycin, at a level to provide 15 mg. daily of aureomycin per 100 pounds of body weight. Aureomycin prevented scours and was accredited with increased rate of growth. Neumann *et al.* (1951) reported that aureomycin reduced or destroyed the vitamin B_{12} synthesizing microorganisms in the rumen. However, indications were that adequate numbers of microorganisms were present, but the types of organisms present were much less diverse.

Colby *et al.* (1950) reported on antibiotic supplements for fattening lambs. One hundred mg. of either aureomycin, penicillin or streptomycin caused weight loss or decline in rate of growth.

Experimental Methods

Lambs originating in the range states and native lambs were used in these experiments during the summer and fall of 1951. All the lambs were hand fed and effort was made at all times to have the lambs on full feed. A standard lamb fattening ration consisting of alfalfa hay and shelled corn, plus .2 pound of soybean meal was fed per lamb daily. Due to the lambs going off feed there were times when no grain was fed for one or two feedings.

The lambs were weighed individually and sorted into the respective

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[2] Aureomycin donated through courtesy of Lederle Laboratories, Pearl River, New York.

lots on a random basis. The lambs were fed under cover and fresh water, salt and a salt-bone meal mix were available at all times.

Aureomycin, in the form of Aurofac A (containing 1.8 mg. of Vitamin B_{12} and 1.8 grams of aureomycin per pound) was incorporated in soybean meal pellets. Various levels of aureomycin were used in these experiments. In the first experiment 3 pounds of Aurofac A was mixed with 100 pounds of soybean meal. In the last three experiments 4 pounds of Aurofac A was mixed with 100 pounds of soybean meal. In order to use the same aureomycin fortified pellets for experiments II, III and IV and yet provide aureomycin at a lower level it was necessary to feed half of the protein supplement as plain soybean pellets and half as aureomycin fortified pellets. With this ratio the pellets when fed at the rate of .2 pound per lamb daily supplied 7.2 mg. of aureomycin. Straight aureomycin fortified pellets fed at the rate of .2 pound per lamb daily supplied 14.4 mg. of aureomycin. Aureomycin was fed so as to supply the following rates: experiment I, 10.8 mg.; experiment II, 14.4 mg.; experiment III, 7.2 mg.; experiment IV 7.2 and 14.4 mg. per lamb daily.

Since there was considerable difference in the quality of lambs used in the various experiments and different levels of aureomycin were fed, each experiment will be discussed separately.

Results and Discussion

The results of experiment I are given in table 1. These lambs were western lambs of approximately fourteen months of age and were of just fair quality. Previous to being placed on this experiment they had been receiving soft ear corn and alfalfa hay.

After 58 days of feeding during the summer months, the aureomycin supplemented lambs, lot 2, appeared more thrifty but their rate of gain was not significantly different from lot 1, the controls (.38 pound and .37 pound average daily gain, respectively). There was, however, a decided difference in the feed efficiency of the two groups of lambs in favor of the aureomycin fed lambs.

In experiment II, 44 native lambs were used. Twenty-two of these lambs received 14.4 mg. of aureomycin per lamb daily and the balance were used as controls. These two lots of lambs were fed equal amounts of the basal ration. In this trial the control lambs gained .40 pound per lamb daily and the aureomycin fed lambs gained .34 pound during a 64 day feeding period. The feed required per 100 pounds of gain

TABLE 1. EFFECTS OF AUREOMYCIN FED AT TWO LEVELS IN FOUR EXPERIMENTS TO FEEDER LAMBS

	Experiment I June 30-Aug. 27, 1951		Experiment II Aug. 6-Oct. 9, 1951		Experiment III Sept. 5-Dec. 19, 1951		Experiment IV Sept. 18-Dec. 18, 1951		
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 3
Number of lambs	17	17	22	22	20	20	27	27	27
Aureomycin fed, mg.	none	10.8	none	14.4	none	7.2 mg.	none	7.2 mg.	14.4 mg.
Average initial weight, lbs.	82.	80.	67.	68.	53.	55.	64.	65.	65.
Average final weight, lbs.	103.	102.	93.	90.	71.	82.	101.	101.	99.
Gain per lamb, lbs.	21.	22.	26.	22.	18.	27.	37.	36.	34.
Daily gain per lamb, lbs.	.37	.38	.40	.34	.17	.26	.41	.40	.37
Days on feed	58	58	64	64	105	105	91	91	91
Death loss	1	1	0	0	0	0	7	3	2
Average daily feed consumption									
Shelled corn, lbs.	1.39	1.33	1.30	1.30	.72	1.05	1.20	1.23	1.26
Alfalfa hay, lbs.	1.78	1.78	1.50	1.50	1.29	1.37	1.43	1.48	1.48
Soybean meal, lbs.	.17	.17	.20	.20	.20	.20	.20	.20	.20
Feed per 100 pounds of Gain									
Shelled corn, lbs.	377.	346.	322.	381.	424.	404.	297.	306.	339.
Alfalfa hay, lbs.	482.	464.	371.	441.	756.	525.	353.	370.	399.
Soybean meal, lbs.	47.	45.	50.	59.	121.	83.	47.	50.	54.

was considerably less for the control groups as compared with the aureomycin fed group. Results of this experiment are given in table 1.

In experiment III, 40 native lambs were divided equally into two groups, one group served as the control and one group received 7.2 mg. of aureomycin per lamb daily. These lambs were of very poor quality and had made poor gains during the pasture season. They were all wormed and fed the basal ration for 105 days. During the course of the experiment every effort was made to get the lambs on full feed. While neither lot consumed very much grain the aureomycin fed lambs consumed .33 pound more corn and about .08 pound more hay per lamb daily. Very likely this largely accounted for the greater gain made by the aureomycin fed lambs as compared to the control group.

At no time during the 105 day feeding period did the treated group exhibit any adverse effects from prolonged aureomycin feeding. The results of experiment III are given in table 1.

In experiment IV, 81 good quality Idaho feeder lambs were randomly allotted into three groups. Lot 1 served as the control, lot 2 received 7.2 mg. of aureomycin per lamb daily and lot 3 received 14.4 mg. of aureomycin per lamb daily.

These lambs weighed about 65 pounds when placed on experiment and were fed heavily on grain for a portion of the feeding period. For a period they were receiving about 1.8 pound of concentrate per lamb daily. As expected, severe death loss resulted. The results of this trial (table 1) show however, that the death loss was considerably higher in the control lot than in either lots 2 or 3. This suggests that aureomycin may have some favorable effect on curtailing death loss due to overeating. Post-mortem examination by the college veterinarian was made on all lambs dying during this experiment. The cause was diagnosed as enterotoxemia. It might be added at this point that several commercial lamb feeders who are using aureomycin in their feeding rations have observed a decrease in scours, decrease in death loss particularly among the "hospital lambs" and a general improvement in thrift.

The rate of gain of the lambs in lots 1, 2 and 3 was .41 pound, .40 pound and .37 pound respectively. The results of this experiment are given in table 1.

In both experiment II and IV, the group of lambs receiving 14.4 mg. of aureomycin per lamb daily made slower daily gains and required more feed per 100 pounds of gain than the control. However, in experiment I, 10.8 mg. of aureomycin did not affect the rate of gain adversely. Aureomycin fed to unthrifty low quality lambs at the level of 7.2 mg.

increased the rate of gain .1 pound per day in experiment III. In experiment IV good quality lambs receiving 7.2 mg. aureomycin gained slightly slower and required slightly more feed per 100 pounds of gain, table 1). Further work will be necessary to determine the optimum level of aureomycin.

Summary and Conclusions

Four feeding experiments involving 199 native and western lambs were conducted to study the effects of aureomycin in lamb fattening rations. These lambs were all full fed a basal ration consisting of shelled yellow corn, alfalfa hay and soybean meal. Aureomycin in the form of Aurofac A was incorporated in the soybean meal and fed at levels of 7.2, 10.8 and 14.4 mg. of aureomycin per lamb daily.

The findings of these four separate experiments are summarized:

1. Aureomycin may be included in lamb fattening rations at levels from 7 to 14 milligrams over a period of 100 days without causing the lambs to go off feed or increasing the incidence of scouring.
2. Aureomycin in these trials did not stimulate feed consumption to the extent that has been observed in trials with swine. However, in two of the trials it was observed that the aureomycin fed lambs were easier to get on full feed.
3. Aureomycin fed at the level of 14.4 mg. per lamb daily reduced the rate of gain and feed efficiency in both trials in which it was fed.
4. At the levels fed in these experiments, aureomycin did not give complete protection from enterotoxemia but it did reduce it materially in experiment IV.

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VALUE OF ADDED METHIONINE IN LOW-PROTEIN AND UREA RATIONS FOR LAMBS[1]

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A KNOWLEDGE of factors which influence the utilization of urea nitrogen by ruminants is of particular importance in making efficient use of this compound as a substitute for some of the protein in rations for cattle and sheep. Loosli and Harris (1945) found that the amount of nitrogen retained by lambs fed a ration containing 1.25 percent of urea was definitely increased by the addition of methionine. They reported that an equal amount of sulfur, added as sodium sulfate to the urea ration, was without statistically significant effect. Average values for percentage of dietary nitrogen retained, however, favored the sulfur-supplemented ration over the unsupplemented ration. Their results with methionine added to a urea ration were confirmed in a later study by Lofgreen, Loosli and Maynard (1947). In both of these studies about 40 percent of the total nitrogen in the rations was supplied by urea. The possible effect on nitrogen retention of methionine added to a low-protein ration unsupplemented with urea or one containing adequate protein, supplied entirely by conventional feeds, was not reported. A protein sparing action of methionine has been reported for dogs (Allison *et al.* 1947) and rats (Bush *et al.* 1947) and may also be true for lambs.

Recently Klosterman and associates (1950) reported that methionine supplementation failed to improve the biological value of nitrogen in a low-protein ration of grass hay, beet pulp, sugar and minerals fed to ewes. Likewise, it failed to improve the utilization of nitrogen in corn rations supplemented with urea (Klosterman, Bolin and Dinusson 1951). Improvement was noted in some experiments but not in others, however, when methionine was added to rations containing 10 percent or more of protein supplied by field peas or alfalfa (Klosterman *et al.* 1951).

Garrigus and associates (1950) reported on the growth of wool and gain in body weight made by lambs in trios fed a methionine-deficient

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[2] The authors are indebted to Professor Carl E. Marshall for statistical analysis of the data in this paper.

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ration (basal), this ration supplemented with methionine, and supplemented with elemental sulfur. They found that the basal ration was definitely improved by the addition of methionine, and that differences between lambs fed the basal ration and the sulfur supplemented ration, while not statistically significant, favored the lambs fed the sulfur supplement. That lambs have a dietary requirement for sulfur which can be met, in part at least, by inorganic sulfate has been clearly demonstrated in recent work by Thomas, Loosli, Williams and Maynard (1951).

In the experiments reported here, digestion and nitrogen balance trials were conducted with lambs to investigate the effect of methionine when added to a low-protein ration adequately supplied with sulfur, and when added to the same ration increased in nitrogen by addition of urea.

Experimental

Eight Texas feeder lambs of mixed breeding were used in these experiments. The lambs had been used in a previous digestion trial and were accustomed to the conditions imposed by nitrogen balance studies. Their average weight was 78 pounds.

The low-protein ration was composed of cottonseed hulls 37.5 percent, cottonseed meal 6.6, yellow corn 28.0, corn starch 14.2, brown sugar 10.3, corn oil 1.0, and mineral mixture 2.4. One ml. of high-potency fish liver oil was added to each day's feed. The mineral mixture contained salt and bone meal plus 2 percent of trace minerals furnishing iron, copper, iodine, cobalt, and manganese, and 8.4 percent of sodium sulfate. The percentage chemical composition of the ration on a dry matter basis was protein (N×6.25) 7.2, ether-extract, 2.9, crude fiber 19.0, N–free extract 66.7, and ash 4.2. Supplementation of this ration with methionine consisted of the addition of either 3.0 or 6.0 gm. of DL-methionine to the daily feed allowance.

The urea ration was made by adding 1.05 gm. of a commercial preparation of urea[1] to 100 gm. of the low-protein ration. Its composition, therefore, was essentially the same as that of the basal ration except for the increased percentage of nitrogen. The total nitrogen of the ration, as shown by chemical analysis, was equivalent to 10.19 percent protein, dry matter basis. Urea supplied approximately 30 percent of the total ration nitrogen. Methionine supplementation of this

[1] Du Pont "Two-Sixty-Two" feed compound, a commercial preparation of urea for feeding purposes which contains 42% of nitrogen.

ration in different trials consisted of the addition of 1.6, 2.0, and 3.0 gm. of DL-methionine to the daily feed allowance.

The unsupplemented and methionine-supplemented urea rations were fed in the first set of four trials, followed immediately by five trials in which the corresponding low-protein rations were fed. Each trial involved four lambs, two lambs receiving the unsupplemented ration and two receiving the same ration supplemented with methionine. Urine and feces were collected during ten-day periods preceded by ten-day preliminary feeding periods. Any lamb that refused to consume the entire ration during either period was taken off trial. Other procedures were the same as have been described elsewhere (Gallup, Briggs and Hatfield 1950).

Results

Results are presented in table 1 in the order in which the trials were conducted. Since the results were found to be unrelated to the different amounts of methionine fed, data obtained in similar trials with different amounts have been grouped together.

In trial 11, with two lambs receiving the unsupplemented urea ration and two receiving the same ration supplemented with 1.6 gm. of methionine, nitrogen retention and utilization values were in favor of the supplemented ration, as were also the values for digestibility of organic matter and crude fiber. Nitrogen retention expressed as a percentage of the intake was increased from an average value of 13.2 to 16.3 percent. The retention of nitrogen was somewhat less than had been observed with a similar ration in earlier studies; consequently, in subsequent trials, 12 through 19, the feed allowance was increased from 700 gm. to over 900 gm. per day.

At the higher level of feed intake in trials 12 through 14, lambs on the unsupplemented urea ration retained an average of 2.53 gm. of nitrogen daily as compared to 2.90 gm. for those on the methionine-supplemented ration. Thus, nitrogen retention, expressed as a percentage of the intake, was increased from an average value of 18.2 to 21.0 percent while the biological value expressed as the percentage of absorbed feed nitrogen utilized was essentially unchanged. The increase in the apparent digestibility of nitrogen represented by the difference between 51.8 and 56.3 percent is of particular interest in these results because a similar effect of methionine is indicated in the data of Lofgreen, Loosli and Maynard (1947) and also, although less clearly, in the data of Loosli and Harris (1945). In the experiments of Lof-

TABLE 1. RESULTS OF NITROGEN BALANCE[1] AND DIGESTION TRIALS WITH LAMBS

Ration designation	Urea	Urea plus methionine[2]	Urea	Urea plus methionine[2]	Low protein	Low protein plus methionine[2]
Crude protein (N×6.25) on dry matter basis, percent	10.05	10.09	10.22	10.24	7.15	7.42
Trial numbers	11	11	12–14	12–14	15–19	15–19
Number of lambs	2	2	6	6	9	9
Average lamb weight, lb	74	74	83	83	90	90
Dry matter intake, gm	631.4	631.4	846.1	847.0	882.8	886.7
Nitrogen intake, gm	10.15	10.19	13.84	13.88	10.10	10.52
Nitrogen in feces, gm	4.01	3.90	6.67	6.08	6.57	6.59
Nitrogen in urine, gm	4.80	4.63	4.64	4.90	2.13	2.48
Nitrogen retained, gm	1.34	1.66	2.53	2.90	1.40	1.45
Nitrogen retained, as % of intake	13.2	16.3	18.2	21.0	13.9	13.8
Nitrogen retained, as % of apparent digested	21.8	26.5	35.0	37.0	38.1	35.7
Biological value[3]	61.7	63.9	71.0	70.9	90.3	87.1
Apparent digestibility of protein, %	60.4	61.7	51.8	56.3	34.8	37.2
True digestibility of protein, %	94.6	95.8	85.5	89.8	83.0	83.7
Digestibility of organic matter, %	74.1	75.9	68.3	71.3	61.8	60.2
Digestibility of crude fiber, %	54.1	58.6	40.8	47.7	33.7	30.2

[1] Nitrogen balance data are on a daily basis.

[2] 1.6 gm. of DL-methionine added daily in trial 11, 2.0 gm. in trials 12 and 13, 3.0 gm. in trials 14, 15, 16, and 17, and 6.0 gm. in trials 18 and 19.

[3] Calculated on the basis of metabolic nitrogen = 0.55 gm. per 100 gm. dry matter intake, and endogenous nitrogen = 0.033 gm. per kilogram body weight (Harris and Mitchell, 1941).

green *et al.* (1947) this effect of methionine, accompanied by a decrease in urinary nitrogen excretion, resulted in a significant increase in nitrogen retention. In the present experiments, methionine decreased average urinary nitrogen excretion in only two of the nine trials; consequently, differences in nitrogen utilization, while in favor of the methionine ration, were not sufficiently great to have statistical significance.

An increase in digestibility of ration nutrients when methionine was added is also shown in table 1. Values for digestibility of crude fiber increased from 54.1 to 58.6 percent in trial 11 and from 40.8 to 47.7 percent in trials 12 through 14. Since crude fiber digestion is dependent upon an active rumen flora, these results suggest a favorable effect of methionine on rumen organisms, perhaps by meeting their requirement for this amino acid for maximum rate of growth. Although Loosli and associates (1949) have presented convincing evidence of methionine synthesis by ruminants, the high methionine content of rumen bacterial protein as reported by Johanson, Moir and Underwood (1949) is indicative of the special value of this amino acid in the metabolism of rumen bacteria.

In contrast to the results obtained with the urea ration, addition of methionine to the low-protein ration, from which the urea ration was made, was without effect on most nitrogen utilization values. Increasing the methionine supplement from 3 gm. per day in trials 15, 16, and 17 to 6 gm. per day in trials 18 and 19 did not alter the nature of the results. Differences between the unsupplemented and supplemented rations in protein digestibility values were related to differences in the average protein content of these two rations. The low digestibility of crude fiber in both rations is characteristic of low-protein rations in general. It was increased by the addition of urea to the ration as shown by comparison with the results of trials 12 through 14.

The negligible effect of methionine added to the low-protein ration is in agreement with the report of Klosterman *et al.* (1950). It is quite possible that supplemental methionine has a measurable value for ruminants only when added to rations that are adequately supplied with nitrogen but are poorly supplied with sulfur or the sulfur-containing amino acids.

Summary

A ration containing 7.2 percent of protein (N $\times$ 6.25) supplied by cottonseed hulls, cottonseed meal and corn, and the same ration made

to contain the equivalent of 10.2 percent of protein by addition of urea, were supplemented with DL-methionine and fed to lambs in digestion and nitrogen balance trials. Trace minerals and sodium sulfate were included in all rations. Methionine supplementation at the rate of 3 or 6 gm. per day failed to improve the low-protein ration. When added to the urea ration at the rate of 1.6, 2 and 3 gm. per day, it increased the average digestibility of nutrients and nitrogen utilization; these differences between the unsupplemented and methionine rations, however, were not statistically significant.

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DETERMINING THE DIGESTIBILITY AND METABOLIZABLE ENERGY OF WINTER RANGE PLANTS BY SHEEP[1]

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MOST important nutrients in appraising the value of range forage are: digestible protein, productive energy, phosphorus and carotene (vitamin A). Accurate appraisals of these constituents are needed for determining deficiencies of range forage, compounding supplements for range feeding, and for comparing the value of the various range plants.

Generally, the ranges of the intermountain area do not produce forage that furnishes all the dietary needs of grazing animals during the winter grazing season. For optimum nutrition, supplementary feed must be supplied, and the amount and nature will depend upon the quality and quantity of forage consumed.

Livestock operators in this area prefer to feed home-grown supplements, such as corn, barley and alfalfa which are relatively high in carbohydrates, rather than expensive protein supplements that must be shipped long distances. Therefore, it is important to evaluate the energy-supplying qualities of range forage rather accurately so that feeds produced at home can be used to a maximum.

It is theorized that ewes wintering on the range compared to farm lot sheep require somewhat larger amounts of feed and more energy supplying feeds because they travel in search of food and must maintain body temperatures without the aid of shelter (Morrison, 1936). When the allowance of carbohydrates and fats does not meet the energy requirements, protein may be used for this purpose (National Research Council, 1949). This decreases the efficiency of protein utilization for growth, development of the foetus, and tissue repair. Also, the cost of protein for an energy-supplying substance is materially higher than home-grown grains. Therefore, it is desirable to meet the energy requirements by supplying the cheaper carbohydrate feeds so that costlier protein feeds when added will be used more efficiently in meeting the protein requirements.

[1] The authors acknowledge the aid of David O. Williamson for chemical analyses of plant and fecal material and of Hallie Cox and Billie Dahl in collecting experimental data.

[2] Associate professor, professor of range management; and professor of animal husbandry and chairman Institute of Nutrition, respectively.

[3] Approved by the director February 19, 1952, Logan, Utah.

In evaluating rations or diets of animals on the winter range where climate is cold, metabolizable energy values are suitable but are not in common use. Metabolizable energy is a more accurate index than total digestible nutrients or digestible energy because it takes into account the loss of energy through urine and gas. This is believed of great consequence in appraising the nutritive energy values of range shrubs which are high in essential oils.

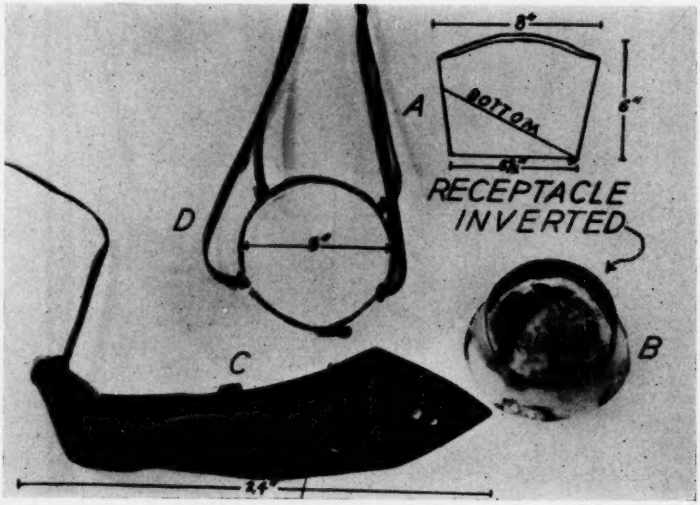

Figure 1. Showing the unassembled parts of a urinal. (A) Cross section of the receptacle. (B) Inverted urinal showing outlet and slope of bottom. (C) 6:50 x 16 inner tube equipped with tractor valve for emptying (D) Adjustable clamp fitting around receptacle after inner tube is in place over receptacle.

Method and Procedure

A method for determining digestibility and metabolizable energy of native forage under range conditions was developed and used during the winter grazing season of 1950–51 and 1951–52. Wether sheep equipped with specially constructed fecal bags and urinals were allowed to graze on ranges composed of a single forage species (figures 1, 2, 3, and 4). This was accomplished by using movable enclosures of about two to four acres which were located on desert ranges in northwestern Utah where plant species occurred naturally in pure stands of a single species.

Four of the more important species of this area are: Black sage (*Artemisia nova*), shadscale (*Atriplex confertifolia*), big sagebrush (*Artemisia tridentata*), and squirreltail grass (*Sitanion hystrix*).

The wethers, varying from 3 to 5 years of age, were allowed an eight-day preliminary grazing period followed by a six-day collection period. During the collection period, forage samples were taken daily by observing individual animals from four to six hours while grazing

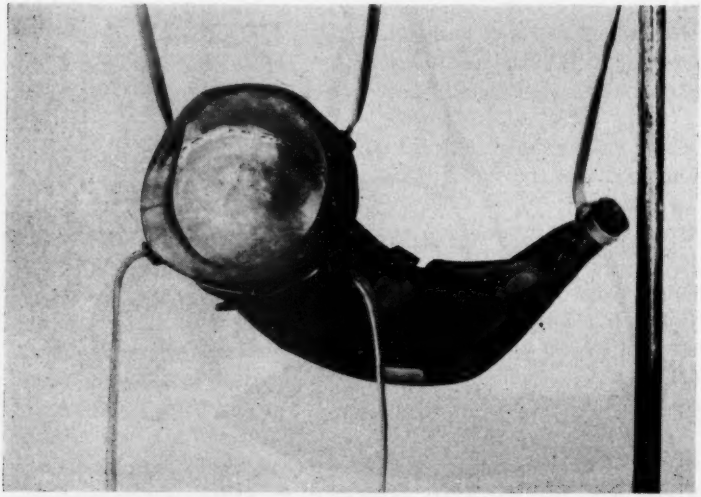

Figure 2. Showing the urinal completely assembled. Note that inner tube stretches over top of urinal and folds back. This provides for a much closer fit to the contours of the animal's body.

and hand plucking forage comparable to the material actually being consumed by the sheep. Many small random plucks were taken over the area as the sheep were normally grazing. When studying mixed stands in previous investigations the before and after method was used (Cook *et al.*, 1950, 1951). The forage samples were composited in duplicate for the entire period and chemically analyzed for constituents included in the modified method of proximate analyses and for gross energy (Cook *et al.*, 1951).

Both the fecal bags and urinals were emptied twice daily during cold weather when freezing was a factor, otherwise, they were emptied only once daily. Both the feces and urine were placed in air-tight containers and, at the end of the period, total output was recorded. Fecal samples

for chemical analysis were composited from the total collection and urine samples for analysis were accumulated for the period by taking 1/10 of the output from each sheep daily.

Fecal material was collected and analyzed as described by Cook *et al.* (1951). The urine samples for analysis were placed in two-quart jars

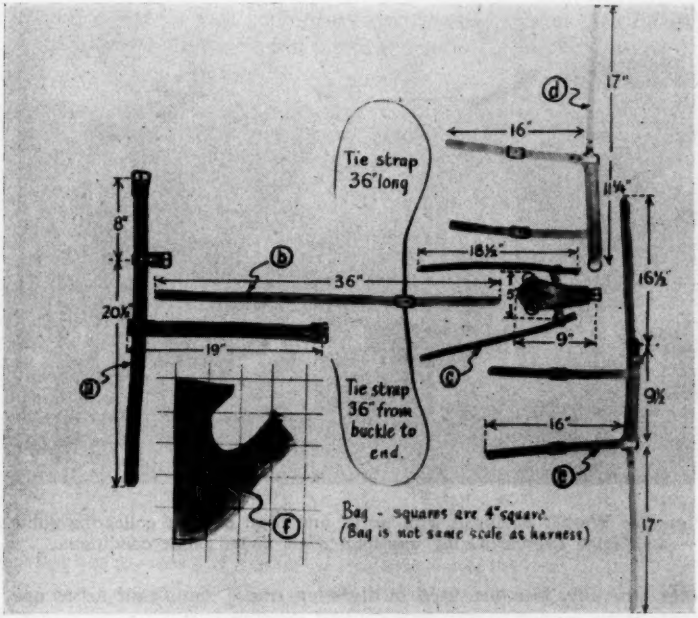

Figure 3. Unassembled parts of sheep harness and fecal bag. This bag and harness fits wethers weighing from 120 to 180 pounds. (a) neck strap; (b) back strap, fastening neck strap and breeching; (c) strap for attaching fecal bag; (d) belly strap, fastening breeching and collar strap; (e) breeching stay; (f) fecal bag equipped with zipper and gathered belly flap. Modified from the original design used by Black and Bratzler, Penn. State College.

containing 3.6 grains of mercury bichloride. In addition, from 10 to 25 cc. of hydrochloric acid were added to keep the urine slightly acid. All urine samples were kept under refrigeration until analyzed for nitrogen, and gross energy. The mercury bichloride and the hydrochloric acid did not significantly affect these chemical determinations.

Daily forage consumption and coefficients of digestibility were determined by the lignin ratio technique as proposed by Forbes and

Garrigus (1948) and Ellis *et al.* (1946). Further details on the technique of determining forage consumption and digestibility under normal grazing conditions are presented by Cook *et al.* (1950–51).

Digestion experiments alone may not measure properly the utilization of energy-supplying materials. In addition to energy losses through the

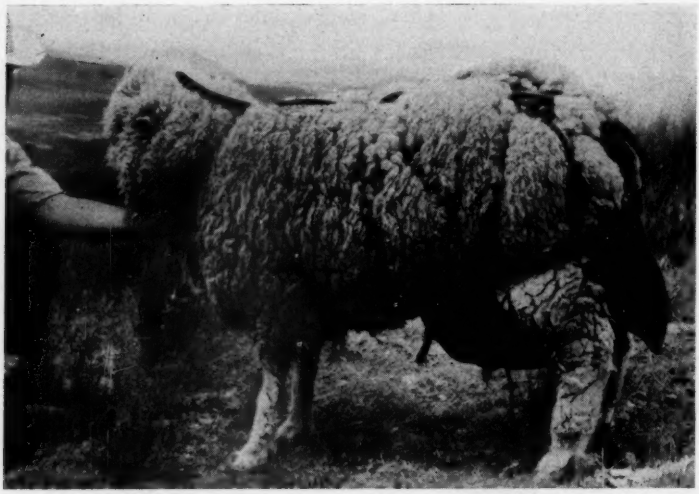

Figure 4. Wether equipped with urinal and fecal bag for collecting solid and liquid excreta during digestion trials under range conditions.

feces (the only loss measured in digestion trials) significant losses are involved in excretion of urine and gasses. Losses through gas result from fermentation in certain segments of the digestive tract. These losses resulting from the formation of methane can be determined directly only in a respiration chamber, however, such losses can be estimated rather accurately from formulas obtained from carefully controlled metabolism trials using the respiration calorimeter.

According to Black[4] methane is produced in proportion to the intake of digestible carbohydrates and can be calculated by the following formula: Methane (grams)$=2.41\ x/100+9.8$ (x represents grams of digestible carbohydrates). Each gram of methane has a calorific value of 13.34 Calories. These factors were used for calculating the energy lost as gas in this study.

The metabolizable energy for each species was calculated by sub-

[4] Black, Alex. Personal correspondence. Penn. State College.

tracting from the gross energy of the feed consumed, the losses in the feces, urine, and methane. The energy loss from urine was corrected for nitrogen equilibrium. The correction for gain or loss of body protein was made according to the procedure outlined by Hamilton *et al.* (1928). For each gram of negative nitrogen derived from the catabolism of body protein 7.45 Calories were subtracted from the gross energy of the urine and for each gram of positive nitrogen balance 7.45 Calories were added to the gross energy of the urine.

TABLE 1. CHEMICAL COMPOSITION OF ALFALFA AND FOUR COMMON RANGE PLANTS CONSTITUTING THE GRAZING SHEEP'S DIET DURING DIGESTION AND METABOLISM TRIAL PERIODS ON A DRY WEIGHT BASIS

Species and year	% Ether extract	% Total protein	% Total ash	% Lignin	% Cellulose	% Other carbohydrates	% Calcium	% Phosphorus
Black sage								
1950	11.1	8.2	5.2	16.1	25.2	37.8	0.82	.15
1951	10.4	8.7	5.8	15.1	17.7	42.3	0.80	.18
Shadscale								
1950	2.9	8.6	25.1	13.9	12.5	37.0	2.54	.07
1951	2.2	5.6	25.6	11.8	17.7	37.1	2.27	.08
Big sagebrush								
1951	8.2	9.0	9.6	16.6	18.5	38.1	0.98	.21
Squirreltail grass								
1950	2.2	3.1	17.0	8.3	40.4	29.0	0.37	.06
Alfalfa hay[1]	3.0	20.5	10.2	6.2	29.1	31.0	—	—

[1] Data from (Hamilton *et al.* 1928) for good quality alfalfa hay.

Results and Discussion

Metabolizable energy is a practical and significant measurement of the value of a forage in satisfying the energy requirements of the grazing animal. The losses of energy through urine and gas are extremely variable for the forage species found on desert ranges of the intermountain area. For this reason, it is impossible to predict accurately the energy supplying qualities of most desert ranges by the use of "total digestible nutrients" or "digestible energy". This is especially true when the foraging animal's diet includes browse species high in the essential oils, such as sagebrush species (*Artemisia spp.*), rabbitbrush species (*Chrysothamnus spp.*) and many others.

The chemical composition of four desert range plants common on the winter ranges of the intermountain area is shown in table 1. These are

compared to good alfalfa hay to show the relative nutrient content. The apparent digestibility coefficients for the nutrients are shown in table 2. The values for 1950 are averages from two wethers whereas the values in 1951 are from seven wethers. As would be expected, the chemical content and the digestibility coefficients for the forage species varied from year to year. The trial areas were widely separated, being

TABLE 2. APPARENT DIGESTIBILITY OF NUTRIENTS PRESENTED IN TABLE 1 AS DETERMINED BY THE LIGNIN RATIO TECHNIQUE COMPARED TO ALFALFA

Species and year	% Ether extract	% Total protein	% Cellulose	% Other carbohydrates	% Total digestible nutrients	% Digestible organic matter	% Digestible energy	% Digestible protein
Black sage								
1950	53.7	52.0	31.6	58.9	45.4	38.0	41.1	4.3
1951	58.9	54.9	25.2	65.8	53.5	45.9	41.9	4.8
Shadscale								
1950	38.9	61.9	27.3	63.5	34.8	33.4	42.5	5.3
1951	7.2	40.4	39.1	60.2	31.9	31.7	34.8	2.3
Big sagebrush								
1951	66.1	42.3	44.2	50.5	43.4	36.6	40.6	3.8
Squirreltail grass								
1950	34.8	0.9	75.9	62.1	50.4	49.5	53.4	0.3
Alfalfa hay[1]								
	51	78	55.8	84.6	61.9	59.9	65.4	16.0

[1] Data from (Hamilton *et al.* 1928) for good quality hay.

40 miles apart, and the 1951 trial periods were somewhat earlier in the season compared to the 1950 trials for the same species. However, all plants were fully mature and in a winter dormant stage.

Gross energy and metabolizable energy values are shown in table 3 along with calorific losses in the feces, urine, and methane. Black sage and big sagebrush are high in essential oils and shadscale contains very little.

Black sage and big sagebrush would be even higher in gross energy and in losses via urine if sheep ate only the current year's growth. Often when sheep first reach the desert ranges, they eat largely current year's growth of the sage species for a week or so. When animals are consuming only current year's growth and seed stalks, the total digestible nutrient values are often as high as 75 percent. Later in the season, they consume many twigs and stems as large as one-eighth of an inch in diameter. This is believed to reduce the burning taste produced by the high content of essential oils in the new growth. When sheep graze

over a sagebrush area the second time, they eat a large quantity of twig stubbs left from the first grazing. Therefore, the chemical content and digestibility are not characteristic of the current year's growth but rather of the portion of the plant consumed by the grazing animals. The total gross energy for black sage and big sagebrush (table 3) are considerably higher than for the other range species. However,

TABLE 3. GROSS ENERGY, ITS LOSSES, AND METABOLIZABLE ENERGY OF SOME WINTER RANGE PLANTS COMPARED TO ALFALFA

Species and year	Energy, Calories per kilogram of dry matter intake					Percentage losses			
		Losses							
	Total	Feces	Urine	Methane	Metabolizable	Feces	Urine	Methane	Percent metabolizable
Black sage									
1950	5022	2960	819	191	1052	58.9	16.3	3.8	21.0
1951	5180	2994	932	217	1037	57.8	18.0	4.2	20.0
Shadscale									
1950	3617	2449	116	170	882	67.7	3.2	4.7	24.4
1951	3390	2207	180	190	813	65.1	5.3	5.6	24.0
Big sagebrush									
1951	4830	2884	599	217	1130	59.7	12.4	4.5	23.4
Squirreltail grass									
1950	3786	1764	95	223	1704	46.6	2.5	5.9	45.0
Alfalfa hay[1]									
	4495	1555	248	254	2438	34.6	5.5	5.6	54.3
Timothy hay[2]									
Early bloom	4563	2007	165	256	2135	44.0	3.6	5.6	46.8
Post bloom	4530	2387	141	267	1735	52.7	3.1	5.9	38.3

[1] Data taken from (Hamilton *et al.* 1928) for good quality alfalfa hay.
[2] Data from Dr. Alex Black, personal correspondence. Penn. State College.

when the metabolizable energy values are compared it is noted that squirreltail grass is markedly higher. This difference results primarily from the low calorific loss in the urine for the grass species compared to the sagebrush species. The losses of energy through urine were from 12.4 percent to as much as 18 percent for the sagebrush species and only 2.5 percent for the grass species. There is also some difference in the digestible energy values in favor of the grass species. This results from the comparatively high content of cellulose in grass as shown in table 1 and the relatively high digestibility coefficients for cellulose and other carbohydrates (table 2). This, likewise, accounts for the rather high total digestible nutrients found in grass. The two sagebrush species are high in ether extract and likewise the digestibility of this fraction is comparatively high for these species. However, it is known

that a large percentage of the ether extract in sagebrush species is composed of essential oils which are readily digested (Guenther, 1948, 1949). Many of these oils are esters, aldehydes, and ethers that yield energy when ignited in a bomb calorimeter which explains the high gross energy values for the sagebrush species.

It is believed that the essential oils after they are digested are polymerized in the liver or kidneys and passed off in the urine as complex substances. This is suggested since the ether extract content of the urine does not account for the increased calorific loss through the liquid excreta for species high in essential oils and, in addition, volatile oils are not detected in the urine when heated.

TABLE 4. ENERGY VALUES COMPARED ON THE BASIS OF GROSS ENERGY, TOTAL DIGESTIBLE NUTRIENTS, DIGESTIBLE ENERGY, AND METABOLIZABLE ENERGY

Species	Gross energy	T.D.N.	Digestible energy	Metabolizable energy
	(Cal./kg.)	(percent)	(Cal./kg.)	(Cal./kg.)
Black sage	5101	49.5	2124	1044
Shadscale	3503	33.4	1174	847
Big sagebrush	4830	43.4	1948	1130
Squirreltail grass	3786	50.4	2022	1704
Alfalfa hay[1]	4495	61.9	2940	2438

[1] Data from (Hamilton *et al.* 1928) for good quality hay.

As shown in table 4, gross energy, total digestible nutrients, and digestible energy values do not accurately measure the energy supplying qualities of most desert range plants. It can, however, be stated that mature grasses are comparatively high in energy regardless of the calculation used but are low in most other respects. Browse plants are comparatively high in protein and phosphorus but are relatively low in energy-supplying constituents. Although individual browse plants are deficient in some nutrients, as a group they are considered excellent winter forage for grazing animals from the standpoint of an economical winter roughage.

Good alfalfa hay appears to be a much better feed in most cases than winter range forage (tables 1, 2 and 3). However, Armsby (1925) gives slightly lower metabolizable values for alfalfa hay. In this case, alfalfa was given a total gross energy value of 4354 Calories per kilogram of dry matter which contained only 44.9 percent metabolizable energy or 1954 Calories of metabolizable energy per kilogram of dry

matter. In this same publication, the average metabolizable energy value for the more common farm roughages was 1800 Calories per kilogram of dry matter. Timothy hay (table 3) in the post bloom stage yielded about the same amount of metabolizable energy as the desert grass. Thus the desert grass compares favorably with farm roughages in energy-supplying qualities.

The metabolizable energy values often are expressed as Calories per kilogram of digestible organic matter or per kilogram of total digestible

TABLE 5. METABOLIZABLE ENERGY VALUES COMPARED ON THE BASIS OF DRY MATTER INTAKE, DIGESTIBLE ORGANIC MATTER, AND TOTAL DIGESTIBLE NUTRIENTS IN VARIOUS FORAGES

Forage species eaten and year	Per kg. dry matter intake	Per kg. total digestible nutrients	Per kg. digestible organic matter
	(Calories)	(Calories)	(Calories)
Black sage			
1950	1052	2317	2768
1951	1037	1938	2259
Shadscale			
1950	882	2534	2641
1951	813	2549	2565
Big sagebrush			
1951	1130	2604	3087
Squirreltail grass			
1950	1704	3381	3442
Alfalfa hay[1]	2438	3938	4070

[1] Data from (Hamilton *et al.* 1928) for good quality hay.

nutrients. These values for the range species studied are shown in table 5. Values presented by Armsby (1917) show that roughages produce about 3600 Calories per kilogram of digestible organic matter. This again shows that mature grass compares favorably with most common feed roughages as a source of energy.

It was suggested by Hamilton *et al.* (1928) that metabolizable energy expressed on the basis of Calories per kilogram of total digestible nutrients was less variable than when expressed as Calories per kilogram of digestible organic matter. With range plants, the opposite was found (table 5). The metabolizable energy values expressed in units of digestible organic matter are less variable because of the influence of the essential oil content of the plant upon gross energy values and total digestible nutrients. The wastage of energy through urine is not considered in calculating total digestible nutrients, there-

fore, when the digestible ether extract is multiplied by 2.25 considerable error is introduced into the determination of energy values.

Because of the limited use of metabolizable energy values, there has been no attempt to establish recommended allowances for practical application. However, Armsby (1925) reviewed several respiration and digestion experiments using sheep and summarized the results. From these calculations, the cost of maintenance under controlled conditions for a 130 pound sheep based on surface area was approximately 2111 Calories of metabolizable energy per day or about 1371 Calories per kilogram of feed intake.

A more detailed study by Ritzman and Benedict (1930) which dealt with fasting catabolism trials with sheep gave slightly higher figures for the metabolizable energy requirements than those presented by Armsby (1925). Ritzman and Benedict (1930) found that the standard metabolism values obtained in their study with mature sheep represented a uniform heat output which closely approximated 17 percent above the basal requirements. Standard metabolism represented the metabolism 24 hours after food ingestion and included a 2 percent stimulus from food which prevailed at 24 hours after food intake and 15 percent for the effort of standing. They further showed that on the basis of liveweight the heat produced is on the average about 11 to 24 percent lower after a 24 hour fasting period than occurs immediately after eating, and that the difference between the first and second fast day is on the average slightly less than two percent. In these studies it was concluded that the standard metabolism for pregnant ewes varied from 30 to 38 Calories per kilogram liveweight. When 18 percent is added for heat increment the values are 37 to 46 Calories per kilogram live weight, or 2182 to 2712 Calories of metabolizable energy daily for a 130 pound ewe. If it is assumed that a 130 pound ewe during gestation consumes 3.6 pounds of dry matter (National Research Council 1949) then the forage should contain from 1339 to 1664 Calories of metabolizable energy per kilogram of dry matter. These figures, of course, do not allow for unsheltered conditions or for travel in search of food under winter range conditions. It is thus apparent that most desert shrubs are somewhat deficient in energy, while grasses are relatively high in energy-supplying qualities.

Summary and Conclusions

The conventional method of appraising the feeding value of common farm lot feeds is not suitable for range forages. This is particularly true when dealing with energy values.

A field method for determining the digestibility and metabolizable energy of native forage under range conditions was developed and used on the winter ranges of Utah during the grazing seasons 1950–51 and 1951–52. The method consisted of grazing wether sheep equipped with fecal bags and urinals in temporary enclosures on pure stands of important desert plants.

Forage was sampled by observing individual sheep while grazing and hand plucking forage comparable to the material actually being consumed. By use of the lignin ratio technique, daily consumption of dry matter and digestibility coefficients were determined. Urine was collected and analyzed for nitrogen, and gross energy, thus, the metabolizable energy could be calculated.

Species of forage high in essential oils had high gross energy and high total digestible nutrient values but were relatively low in metabolizable energy. These comparatively low metabolizable energy values were obtained because of the extremely large quantity of energy lost through the urine. The essential oils were responsible for high gross energy values and high total digestible nutrients but were eliminated largely through the urine and did not represent available energy for animal use. Therefore, an accurate appraisal of the nutritive energy furnished by many range forage plants can be assessed only by determining their metabolizable energy values.

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SOME WOOL CHARACTERISTICS OF THE OSSIMI SHEEP

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MOST of wool crop in Egypt is taken from the Ossimi sheep which have a white fleece with a reddish-brown face. Very little is known about the characteristics of Egyptian wool.

Variations in fiber diameter have been shown to exist (Pohle, Keller and Hazel, 1944). Wolf, Dawson and Pohle (1943) found that the variability in the density of wool fibers was most in the shoulder region and least in the hip region. After a comprehensive study on Shropshire sheep, Hardy and Wolf (1947) concluded that fiber density was greater at 20 weeks of age than at 52 weeks for both shoulder and hip regions. Grandstaff and Wolf (1947) found significant differences in kemp percentage between ages, years and lambs within years.

The purpose of the present investigation was to study the normal process of wool growth and the following characters of the fleece; mean fiber length, density of fiber and the percentage of the weight of kemp.

Materials and Methods

This work has been carried out in the animal breeding department of the Faculty of Agriculture, Giza. Ten Ossimi ewes, about nine months old, were chosen at random from the department's flock. They were born from October 14 to October 26, 1948.

A square containing four square centimeters of area was tattooed on each of the shoulder, back, hip and britch. Samples of wool were cut from these regions close to the skin by fine scissors. A sample from each square was taken every three months. The dates of sampling were: September 22, 1949 for summer growth; December 22, 1949 for autumn growth; March 24, 1950 for winter growth and June 23, 1950 for spring growth. The ewes were not bred during the course of the experiment.

The samples of wool were washed in a 60° C. solution of hot water containing 15 percent soap and one percent sodium bicarbonate. Then they were washed by hot water only, and finally with ordinary water. Samples were dried in an electric oven at 100° C. for six hours. Then they were conditioned in the humidity room at a relative humidity of 65 percent for three days. The samples were weighed in the same room and were used for estimating the weight of wool.

In estimating the mean diameter of the fibers, the mean length of fibers and the density of fiber population, samples from the shoulder and hig regions only were used. In both estimations, the methods used by Galpin (1947, 1948) have been applied. Grades of wool were classified after Davenport and Ritzman (1926). For estimating the percentage of the weight of kemp, the ewes used were chosen from the same Ossimi flock. The method used by Roberts (1926) was applied for this purpose. The methods used for the analysis of data were after Croxton and Cowden (1948).

Results and Discussion

Diameter of wool fibers

The shoulder region had a finer wool than the hip region since the mean diameter of fibers for the four seasons was 35.0 ± 0.31 microns and 39.3 ± 0.31 microns for the shoulder and hip regions respectively. This is in agreement with Berge, Sande and Brekke (1944). Although the shoulder region was found to have finer wool than the other regions, only about 5.5 percent of its wool could be classified as fine wool in the Ossimi. The mean diameter of the fibers of both shoulder and hip regions was 37.2 ± 0.22 microns. It is clear from results obtained by Lang (1944) in Merino and Kammlade (1947) in Suffolk that the Ossimi has a definitely coarse wool.

The diameter of the wool is not uniform all over the fleece, since there was a statistically significant difference in diameter of wool between shoulder and hip regions. The individuals also showed great variability in the diameter of fibers. This is in agreement with Pohle and Schott (1943) and Pohle *et al.*, (1944). There was a gradual increase in diameter from summer (11 months of age) up to spring (20 months of age) as shown in figures 1 and 2. This effect may be due to the advance of age rather than to season. The diameter of wool fibers was less variable during winter than in any other season. This may be attributed to the fact that winter in Egypt helps the growth of wool owing to the favorable climatic and nutritional conditions.

Length of wool fibers

The fibers of the shoulder region were shorter than those of the hip region, i.e. the fiber length averaged 34.8 ± 0.73 mm. and 38.6 ± 0.73 mm. for the shoulder and hip regions respectively for the four seasons. The average fiber length for both the shoulder and hip regions was

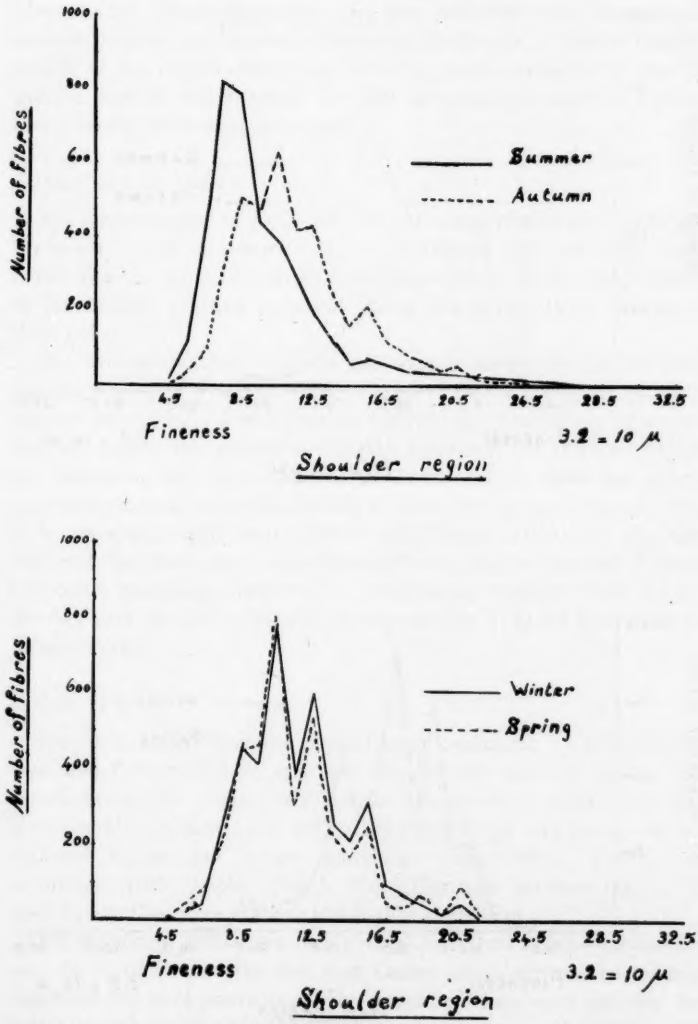
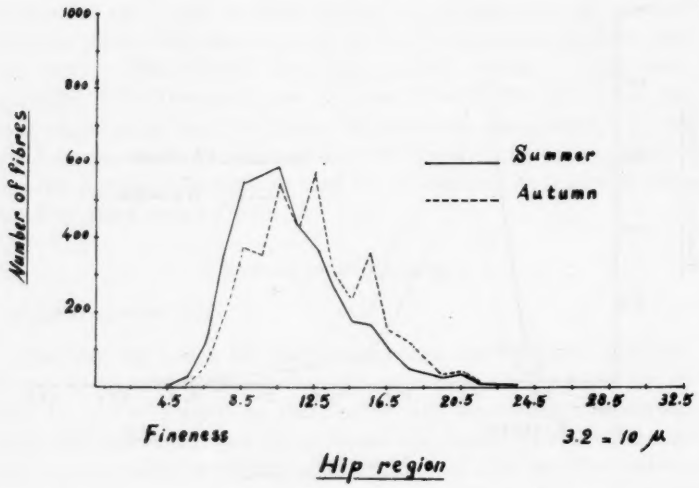


Figure 1. Frequency distribution of diameters of wool fibers of the shoulder region in the four seasons.



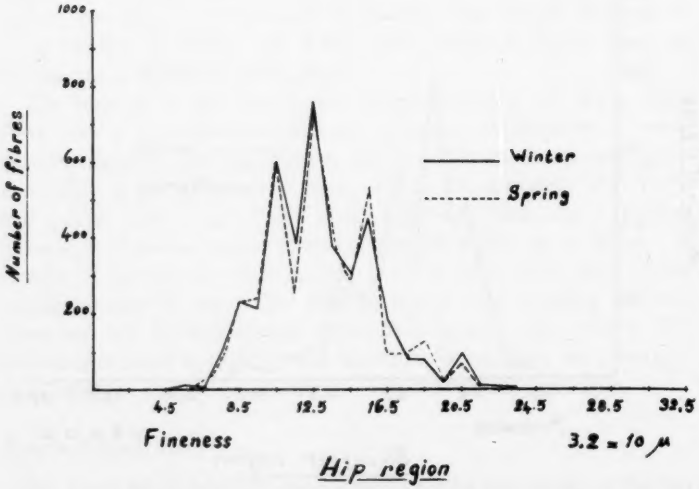


Figure 2. Frequency distribution of diameters of the wool fibers of the hip region in the four seasons.

36.7±0.51 mm. The ewes at the same age differed in the average length of wool fiber. These differences were less noticeable than those found between seasons and regions. Comparing the length of twelve months growth in the Ossimi, which was found to be six inches, with that of Merino, Suffolk and Romney, it could be concluded that the Ossimi sheep belong to the long wool type.

Density of wool fibers

The mean number of fibers for the whole experiment was 1384±21 fibers per square centimeter which is considered very low when compared with that of the European breeds reported by Wolf *et al.*, (1943) in Rambouillet yearling ewes and Hardy and Wolf (1947) in Shropshire sheep.

The average number of fibers per square centimeter for the four seasons was 1343±29 and 1425±29 fibers for the shoulder and hip regions respectively. The difference between the number of fibers for the two regions was statistically highly significant per tattooed square and significant per square centimeter. There was no significant difference between seasons in the number of fibers per tattooed square. This is in agreement with Goot (1945) and Galpin (1948). It was also observed that there was a significant difference in the number of fibers per square centimeter. This could be explained as Galpin (1948) did, by the fact that the skin expanded but the number of fibers was stable at an earlier age.

Weight of wool

The back region gave the highest wool production (4.871±0.1008 mgs. and 0.8566±0.0295 mgs. per day for the tattooed square and square centimeter respectively), while the shoulder region gave the lowest yield (2.872±0.1008 and 0.5173±0.0295 per day in mg. for the tattooed square and square centimeter respectively). This is in accordance with Galpin (1947). The differences between regions in wool production were statistically highly significant.

The significant differences of individual variations in wool production may be attributed to the fact that Ossimi sheep are not adequately improved for wool production. The weight of clean wool per day for both tattooed square and square centimeter was not constant but varied from season to season. The maximum wool production was shown to take place in winter both for tattooed square and square centimeter. Wool production was lowest in summer and spring, especially in the

weight of tattooed square. This may be due to the effect of abundant green fodder during winter, or to the intense heat, shortage of green fodder and the existence of harmful insects in summer. The effect of these adverse conditions seems to persist up to autumn when the increase in weight of wool was barely significant.

There was a drop in wool production in spring season. This can be taken as an indication that the variation in the weight of wool is due to a seasonal effect rather than to age. Furthermore, when the weight of wool was computed for the square centimeter, to eliminate the effect of skin expansion which is a function of body growth, this seasonal variation still persisted.

Kemp Fibers

The mean percentage of the weight of kemp for the whole experiment was 5.8 ± 1.21 percent and 11.8 ± 1.21 percent for the shoulder and britch regions respectively. The mean percentage for the two regions pooled together was 8.8 ± 0.87 percent (figures 3, A and B). This percentage is high in the Ossimi as compared with that of the improved wool breeds. Selection against this character should be carried out immediately among the Ossimi sheep.

Summary

The wool characteristics have been examined on 10 Ossimi ewes in each of the four seasons of the year.

1. The mean diameter of fibers for the shoulder and the hip regions was 35.0 ± 0.31 and 39.3 ± 0.31 microns respectively.
2. The variability of the diameter of wool fibers was lower in winter than any other season.
3. The mean number of fibers for the shoulder and hip regions was 1343 ± 29 and 1425 ± 29 fibers per square centimeter respectively.
4. The average length of fibers for the shoulder region was 34.8 ± 0.73 mm., while it was 38.6 ± 0.73 mm. for the hip region.
5. The maximum wool production took place in winter, while the minimum was in spring (0.8411 ± 0.0295 and 0.5938 ± 0.0295 mg. per square centimeter per day respectively).
6. The back region gave the highest wool production (0.8566 ± 0.0295 mg. per square centimeter per day).
7. The percentage of kemp for shoulder region was 5.8 ± 1.21 percent, while it was 11.8 ± 1.21 percent for the britch region.

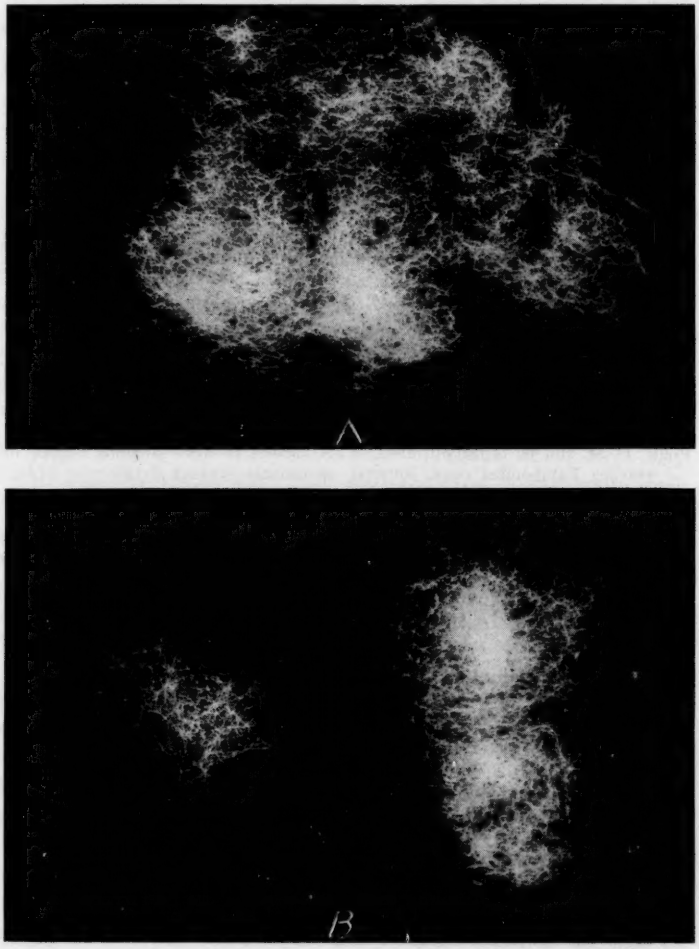


Figure 3. A—Sample of Ossimi Wool.
B—Same sample separated into kemp (left) and non-kemp fibers (right) (16.2 percent kemp).

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THE EFFECT OF NUTRITION UPON WOOL PIGMENTATION[1]

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THE occurrence of banded wool has been reported by several investigators. Boyd (1930) reported the occurrence of color banding in the fleeces of five different sheep. She observed several different types of banding and noted that sheep which produced banded fleeces always began life as colored lambs. Boyd postulated that since banding took place with regularity over the whole fleece, it must in some way be related to a definite physiological condition of the body as a whole, although she thought it to be primarily genetic in origin. Nichols (1927) reported on the occurrence of dark fibers in the Suffolk fleece; he theorized that there are thresholds of irritation for inhibiting factors of pigment production which he thought could be affected by temperature.

A rather common occurrence in some breeds of sheep (for example Hampshires and Suffolks) is the birth of lambs which have very darkly pigmented wool when born and later grow normal white wool. Cross sections of such wool on the flesh side, point of change, and outer tips are shown in figure 2, G, H, and I. Since it is known that the vitamin A levels of new-born lambs are very low, (Weir, *et al.*, 1949), and because vitamin D, which is also a fat soluble vitamin, is probably low, it is possible that either of these vitamins may be responsible for the dark pigmentation.

More recently, Glazener, *et al.*, (1946) demonstrated that diets deficient in vitamin D caused an abnormal blackening of the feathers of New Hampshire chicks. Glazener and Briggs (1948) studied the effects of a vitamin D deficiency on five different breeds of chicks. They showed that there is a breed difference in the abnormal blackening of feathers during a period of vitamin D deficiency. Lillie and Bird (1949) confirmed the work of Glazener and Briggs (1948) showing a difference between breeds and possibly between strains of the same breed.

Previous work with lambs at this station showed that several vitamin A depleted lambs of Hampshire origin produced abnormal amounts of melanin during the latter stages of deficiency. These lambs had been fed a carotene deficient ration and were confined in a shed out of direct contact with sunlight. The fleece of number 176 was found to be distinctly banded when sheared (figure 1, A). The fleece in this

[1] Publication approved by the Director of the North Dakota Agricultural Experiment Station.

[2] The authors gratefully acknowledge Wyeth, Incorporated, Philadelphia, Pennsylvania, for furnishing the vitamin A for these studies.

Thanks are also given to Dr. Glen C. Holm, Veterinarian, N.D.A.C. for the photography.

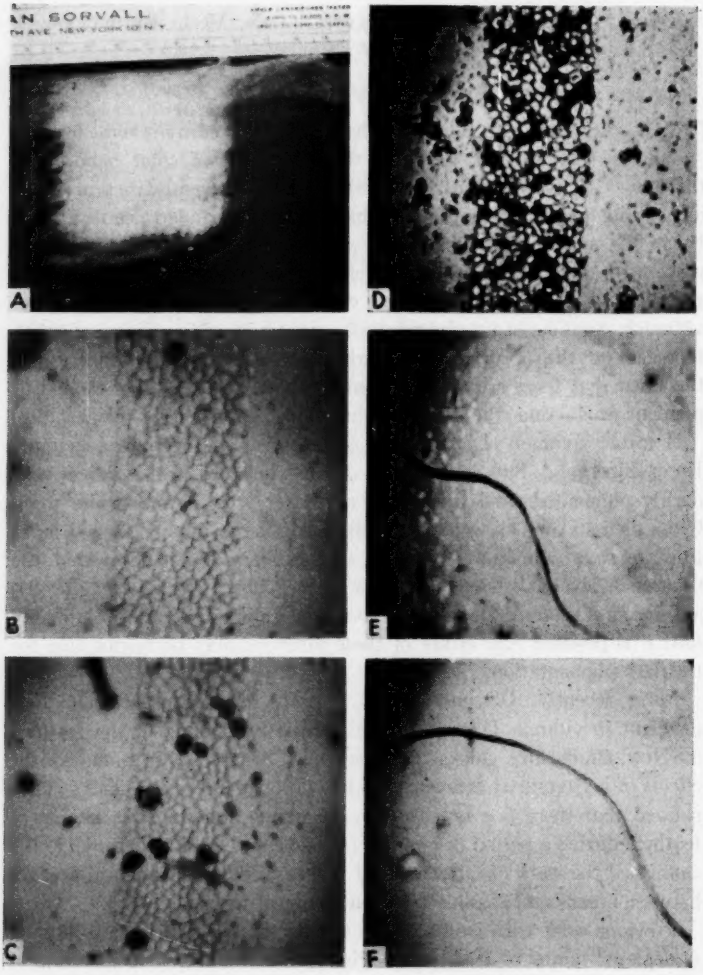

Figure 1.

case was white on the outer portion and black on the flesh side. It was noted in individual fibers that some which were normally black turned white and vice versa. Figure 1, E shows the change from white to

black. Figure 1, F shows the change from black to white. Cross sections of wool tufts on either end and at the point of change are shown in figure 1, B, C, and D.

Experimental Procedure

Avitaminosis A was suspected to be the causative factor involved in the wool pigmentation reversal in ewe number 176. In March, 1950, ewe 176 was given a ration containing sun-cured alfalfa hay to determine whether a change from black to white could be produced. This change did occur as shown on figure 2, J, and the outer fleece shown in figure 2, K and L.

Nine lambs of mixed origin, plus ewe 176, were placed on a carotene deficient ration in June of 1950. The ration consisted of water, wheat straw, beet pulp, soybean oil meal, and a mineral mixture consisting of trace mineralized salt plus bone meal. They were confined in the same shed used in the first experiment and were kept out of direct contact with sunlight, although sunlight was admitted through an open door and through several open windows.

The genetic make-up of the expermiental lambs was as follows. Five were purebred Hampshires, two were white Columbia-type lambs that were sired by a black ram and two were sired by a Delaine-Merino ram and were out of Rambouillet ewes.

The fleeces of the lambs were inspected at the start of the experiment and all fleeces were considered normal for their respective breed types. Blood sera samples were analyzed for vitamin A at varying intervals to determine the extent of avitaminosis A. Gross deficiency symptoms and any fleece changes were noted periodically.

Each lamb was individually treated with either water soluble vitamin A injected intravenously or a vitamin A and D supplement administered by stomach tube after pigmentation changes were noted in the wool fibers of several of the lambs.

Discussion and Results

Analyses of blood sera indicated that the lambs were becoming vitamin A deficient by January of 1951 and in several cases, gross deficiency symptoms were noted. Examination of two rams which were in the group revealed that atrophy of testicular tissue was taking place. Blood sera vitamin A levels were noted to fluctuate throughout the

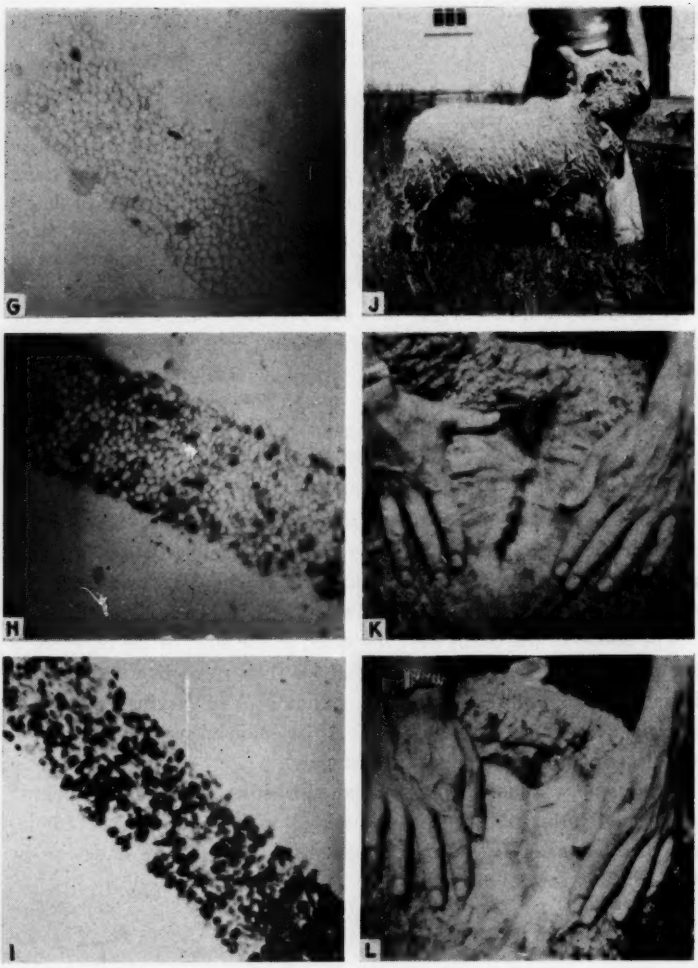

Figure 2.

period of deficiency (table 1). Most levels, however, remained near 30 I. U. per 100 ml. which Eveleth and co-workers (1949) considered a deficient level.

Examination of the wool of the lambs in February of 1951 showed that several of the fleeces of Hampshire lambs were beginning to show a noticeable darkening near the skin. This condition was more pronounced by April, 1951. Photographs of ewe 176 were made in May, 1951 (figure 2, K and L). These photographs of shoulder and rump areas show the fleece changes that accompanied a vitamin deficiency. Color changes were general over the body area.

All sheep were treated with either injectable vitamin A or a vitamin A and D mixture administered orally on June 2, 1951. This treatment

TABLE 1. BLOOD SERA VITAMIN A LEVELS

Lamb No.	I. U. of vitamin A per 100 ml.			Fleece Changes[1]
	2–16–51	4–11–51	5–24–51	
176	17	32	27	+
491	55	35	32	0
1505	45	42	30	0
1514	27	18	23	+
1553	27	18	35	0
1576	35	30	30	0
1580	30	30	42	+
1597	33	40	30	0
1603	30	30	28	0
1604	30	30	30	0

[1] 0 indicates no change, + indicates color reversal.

was repeated July 3, 1951. The amounts used were large enough to bring the sera vitamin A well above deficiency levels.

Detailed examination of the fleeces after a period of two months showed that the black wool fibers of one of the Hampshire sheep were changing from black to white. This sheep had been treated with a vitamin A and D mixture. Ewe number 176, which had been treated only with injectable vitamin A, did not respond.

Since no response was seen during a three month period when ewe 176 was treated with vitamin A, she was given massive doses (approximately 45,000 I.U.) of vitamin D orally at bi-weekly periods beginning in September, 1951. A change from black to white in the pigmentation of wool fibers of ewe 176 was noted in November, 1951. Evidentally this was the result of the vitamin D treatment.

Sheep used in this experiment, which were of a genetic make-up different from the Hampshire lambs, did not show any visible fleece changes. In fact, their fleeces remained normal in appearance through-

out the experiment while several of the Hampshires developed weak spots in their wool fibers. This occurred midway through the vitamin deficiency period and resulted in ragged fleeces and a new growth of wool which in most cases contained a heavy concentration of black fibers. This was not observed in every Hampshire lamb, however.

There is some reason to question whether vitamin D was the sole factor responsible for the pigmentation reversal noted in some Hampshire lambs during this experiment. Since the reversal was noted during a period when the lambs were known to be vitamin A deficient and no lambs were treated with vitamin D solely, it is possible that a combination of vitamin A and D was necessary for the changes brought about. It seems probable, however, that vitamin D acts in some manner to cause a reversal of pigment production in some Hampshire lambs.

Summary

1. A reversal of wool pigmentation from white to black and vice versa has occurred in Hampshire sheep fed rations low in vitamins A and D.
2. Vitamin A treatments to depleted sheep of Hampshire origin brought no response in pigmentation pattern.
3. A reversion to original wool pigmentation was noted when vitamin D was administered to lambs previously treated with vitamin A.
4. Sheep of Delaine-Merino X Rambouillet ancestry or Columbia-type sheep that were heterozygous for black showed no pigmentation reversal pattern.
5. There was a difference between Hampshire lambs in their response to rations deficient in vitamins A and D.

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NEWS AND NOTES

The forty-fourth annual meeting of the American Society of Animal Production will be held in the Hotel Sherman in Chicago, Illinois, on November 28 and 29 with a dinner for the honored guest on November 30.

Proposed amendment of Constitution: It is proposed that Article II, Section 4, of the Constitution of the American Society of Animal Production be amended as outlined below:

The original Section read:

"Section 4. Forfeiture.

"A membership or subscripiton is void if not paid by January 1 of the calendar year to which it applies. Reinstatement of membership forfeited because of failure to pay dues takes place automatically as soon as delinquent dues are paid."

The Amended Section now reads:

"Section 4. Forfeiture.

"A membership is void if dues are not paid by January 1 of the calendar year to which they apply. Members whose dues are paid after January 1 but before July 1 of the calendar year shall be assessed one dollar additional. After July 1 reinstatement of membership will be made only on payment of current dues plus a reinstatement fee of five dollars. A person who was once a member may not be proposed as a new member to avoid payment of the delinquency fee.

Subscriptions to the JOURNAL OF ANIMAL SCIENCE shall be on a volume basis only and shall expire with the calendar year unless renewal or notice of intention to renew is received by that time."

It is proposed to amend the above section to read as follows:

"Section 4. Forfeiture.

"A membership or subscription is void if not paid by January 1 of the calendar year to which it applies. Reinstatement of membership forfeited because of failure to pay dues takes place automatically upon the payment of a penalty of one dollar and dues for the current year; numbers of the JOURNAL OF ANIMAL SCIENCE mailed prior to reinstatement will not be a perquisite of membership but can be purchased, when available, at the regular price for separate numbers. A person once a member cannot be proposed as a new member to avoid payment of delinquency penalty.

Subscriptions to the JOURNAL OF ANIMAL SCIENCE shall be on a volume basis only and shall expire with the calendar year unless renewal or notice of intention to renew is received by that time."

This proposed amendment will be discussed and voted upon at the forthcoming meeting of the Society in Chicago.

Ralph W. Phillips, Deputy Director of the Agriculture Division of the Food and Agriculture Organization of the United Nations was awarded an honorary Doctor of Science degree by Berea College in Berea, Kentucky on June 2, 1952.

The annual meeting of the Western Section of the American Society of Animal Production was held June 23 and 24 at the University of California, Davis. Thirty-three scientific papers were presented by members. Officers elected for next year were: President—J. L. Van Horn (Montana); Vice-President—Hubert Heitman, Jr. (California); and Secretary-Treasurer—L. E. Washburn (Colorado). Ninety-three registered for the meetings, but attendance was larger at times due to the meetings of the American Dairy Science Association held June 24 to 26.

The meetings of the Western Section will be held in Fort Collins, Colorado, next summer with Colorado A & M College as the host institution.

O. E. Reed, Chief of the Bureau of Dairy Industry, was this summer given a distinguished service award by the U. S. Department of Agriculture for his outstanding work in the dairy industry.

The third edition of Jull: *Poultry Breeding,* was published by John Wiley & Sons, Inc., N. Y. in April, 1952. The book has been completely revised bringing in the latest information on fertility and hatchability, viability, meat production, and egg production. A new chapter on selection methods has been added.

The Economics of Poulty Management, by Harold E. Botsford, was published by John Wiley and Sons, Inc., N. Y., in May, 1952. An examination is made of the costs of the various operations in poultry management and how these change at various times. The effects of geographic region on these costs are also studied.

A World Dictionary of Breeds Types and Varieties of Livestock, by I. L. Mason was published by the Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England in May, 1952. The breed composition of the livestock of as many countries as possible is analyzed and tabulated with references to the literature. English and foreign synonyms and information about the breed are given.

J. F. Hentges joined the Department of Animal Husbandry and Nutrition at the University of Florida on July 15, 1952. He will do teachnig and research on beef cattle production. He received his B.S. degree at Oklahoma A & M and his M.S. and Ph.D at the University of Wisconsin.

Paul B. Pearson was designated by the Department of State as United States delegate to the Sixth International Congress of Animal Husbandry held in Copenhagen July 9 to 14. He also participated in the Second International Biochemical Congress in Paris the last week in July.

Jorge de Alba, animal husbandman at the Inter-American Institute of Agricultural Sciences, who has been acting as director of the newly organized Escuela Agropecuaria de Sonora, Mexico, returned to the Institute July 1 as head of the Animal Industry Department.

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INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, double-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should contain a clear statement of the purpose of the investigation and its relation to other work in the same field, but long reviews should be avoided. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results, or their relation to some problem of the livestock industry. Each paper should end with a brief summary outlining the points proved in the investigation and their application, or their relation to a livestock problem.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. The author or his institution except in the case of invited review papers, will be required to purchase at least 100 reprints of his paper. There will be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsibility for the charges and for the reprints that will be ordered at that time.

6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts". *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest concerning the members, and other items of interest to the membership. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. L. E. Casida, Department of Genetics, University of Wisconsin, Madison, Wisconsin, or to the publishers: Boyd Printing Company, 372–374 Broadway, Albany 7, New York.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. J. I. Miller, Department of Animal Husbandry, Cornell University, Ithaca, N. Y., or to the publishers, Boyd Printing Company, 372–374 Broadway, Albany 7, New York.

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